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NORMAL EXCRETION OF SEX HORMONES IN CHILDHOOD[1]

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THE GLANDS of internal secretion are of great importance in childhood. Their three major functions are: to stimulate growth, to influence metabolic activities and to regulate physical metamorphosis of adolescence. The rate of growth and the resting metabolic rates (factors largely influenced by the secretion of the endocrine glands) have been discussed by others, but very little work has been done previously in regard to the third function. This investigation deals chiefly with the urinary excretion of the sex hormones, measurements which should indicate the rate of activity of the glands from which these secretions are derived.

Development in normal youth is essentially a problem of normal activity of the pituitary gland and the ability of the peripheral endocrine organs to respond to its stimulation. Laboratory and clinical evidence indicate that without the normal functioning of this particular endocrine organ, the otherwise normal peripheral glands such as ovaries and testicles remain quiescent and unstimulated. The problem, therefore, of normal sex differentiation in growth in childhood is essentially a problem of the stimulation of the peripheral glands by the secretions of the anterior pituitary gland. Alterations in puberty seem to be due usually to abnormalities of the anterior pituitary stimulation and much less frequently to abnormalities of the gonads, the adrenals, or other endocrine glands. This is the reverse of what happens at the menopause, when the pituitary seems to be actively secreting, but the degenerating gonads can no longer respond to stimulating hormones. It is unfortunate that available technics are not now sufficiently accurate to obtain the ideal data for the study of the complete sex mechanisms of childhood. This would include quantitative determinations of blood and urinary levels of the pituitary, adrenal and sex hormones. The small amounts of these hormones found in childhood cannot be measured accurately in the blood at present. The pituitary sex hormones can be measured in the urine of children and their excretion has been studied and here recorded. We are now able to quantitatively appraise the rate of the excretion of the male and female sex hormones in

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the urine. This we have done, substituting quantitative values for the rough objective observations of physical examination. By this method we have learned the relative activity of the sex glands in childhood, and these data are here presented.

Relatively little work has been reported in this field. There have been scattered observations of childhood excretion rates of female and male sex hormones, but no elaborate study has been made.

Single determinations of estrogens were reported in the urine of girls between the ages of 6 and 11 by Zondek and Euler (1) and subsequently by such investigators as Wu and Chou (2), Dorfman and Greulich (3) and Oesting and Webster (4). Frank (5) found evidence of a cyclic excretion of the hormones several months before the menarche in one case. Fluhman (6), using a different technic, made blood determinations on 15 girls ranging in age from 4 to 10. Of eight children 7 years of age or younger, one gave a weak reaction, whereas of 7 children between the ages of 8 and 10, 5 gave a stronger reaction, one a weak reaction, and one was negative.

Androgens in the urine of children have been reported (2, 3, 4, 7, 8, 9). With the exception of our own series (10), these were all single observations on smaller groups of children.

Technics of Analysis

The urinary estrogens and androgens (17-ketosteroids)[2] were extracted by the method of Smith and Smith (11). This consists of preliminary hydrolysis by hydrochloric acid and subsequent extraction with benzol. The estrogens were separated from the 17-ketosteroids, using in principle the method of Gallagher et al. (12). With this technic the 17-ketosteroids which are insoluble in alkali remain in the neutral fraction and are thus separated from the phenolic estrogens which are soluble in alkali (NaOH).

The estrogens were then assayed on inbred pure strain mice (Bar Harbor C57) or rats (Slonaker strain) with a minor modification of the Allen-Doisy method: estrogens are expressed in the tables in I.U. equivalent to crystalline estrone.[3]

Androgens in the urine were not determined biologically, but a colorimetric test for all of the 17-ketosteroids in the urine was made. This includes the androgens such as androsterone and dehydroisoandrosterone, and also includes some other closely related compounds which are not androgenic in biological activity, when they are excreted, but may represent catabolic changes of such compounds. The technic of analysis was that described by Oesting (13), except that certain conditions such as temperature, light and purity of reagents, were kept as uniform as possible. The reaction which was originally described by Zimmerman (14) is based on the ability of meta-dinitrobenzene to produce a color reaction quantitatively with substances containing the $-CH_2CO$ grouping in alkaline alcoholic solution. With the exception of the earliest readings, all titrations were made simultaneously with the Oesting colorimeter and Evelyn photoelectric colorimeter. Since the first readings were made only with the former colorimeter, and although we have been able to correlate satisfactorily the later readings by both technics, we have adopted the original unit (Color Unit) as the standard of measurement for this study. With the method used, which will be described in detail in the near future, one color unit is equivalent to 60 gamma of crystalline androsterone.[4]

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[2] The words androgens and 17-ketosteroids are used interchangeably in this paper. The synonymous use of the words is for style of presentation rather than accuracy of their denotation. Actually the chemical determinations are for the total 17-ketosteroids.

[3] The estrone (Theelin) was supplied for standardization through the courtesy of Dr. E. A. Sharpe of Parke, Davis & Co.

[4] The androsterone which was used to standardize our procedure was generously supplied by Doctors Erwin Schwenk and Gregory Stragnell of the Schering Corporation, and by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc.

Gonadotropic hormone (follicle stimulating hormone, FSH, prolan A) was precipitated from the urine by means of ethyl alcohol, using the original technic of Aschheim and Zondek. Assay was carried out on inbred immature pure strain mice (Bar Harbor C57) between 19 and 21 days old and weighing between 6 and 8 gm. Follicle stimulation in the ovary was used as the criterion of gonadotropic activity in the extract. This method in our opinion, will demonstrate a response not apparent by estimation of ovarian or uterine weight, or by vaginal opening. The material which was precipitated was so concentrated as to detect a minimum of 20 mouse units (M.U.) per 24 hours. One mouse unit is defined as the amount of material, which when injected in 5 equal parts over a period of 48 hours, will produce follicle stimulation in the ovaries of the animal 96 hours after the first injection.

There have been a total of 1100 determinations made in this study, of which 565 were for estrogens (female hormones) 495 for 17-ketosteroids (male hormones) and 40 for the follicle stimulating hormone of the pituitary gland (FSH). These have been made on 104 normal children, of whom 59 were girls. The observations on 41 children with ages varying from 3 to 6 years, have been largely drawn from a group of children enrolled for study in the Department of Child Hygiene of the Harvard School of Public Health (15). A large percentage of the group above six years have come from children in a growth study conducted at a private school; twelve other subjects have been obtained among normal children of doctors.

Inasmuch as an adequate chemical technic for determining 17-ketosteroids was not available during the first year and a half, only single 24-hour urine determinations of the estrogens were made on each child. Then it was obvious that there was an enormous scattering of values obtained in children who were 9 years old or older. This made it seem probable that the excretion of estrogens was not the same every day, but that there might be a cycle of excretion such as is found in the adult. From that time on determinations were largely limited to a smaller group of children who were willing to collect 24-hour specimens twice a week over a period of a month. In this way we were able to obtain a picture of cyclic excretion when it was present, and were able to determine roughly the time of the appearance of the cycle in relation to the later development of the menarche. Obtaining accurate, repeated collections from normal children was difficult. Completeness of all the 24-hour specimens was determined by the creatinine content of the urine, so that each 24-hour specimen could thus be checked in an objective way.[5] As a result, we think that the collections here reported are quite accurate and represent values from a group of children who are, on the whole, probably in better than average physical condition.

RESULTS

The results of this study have been summarized in the accompanying charts and tables, which show the change of excretion levels of FSH and of androgen and estrogen components according to age and sex. Determinations of single 24-hour urinary collections are recorded in the scattergraphs, while consecutive determinations are recorded separately.

The gonadotropic hormone (FSH) was not determined on all cases, but only on 13 boys and 14 girls, whose development put them in the obviously important age group. Tables 1 and 2 show the accumulated data.

In the male no FSH could be detected before the age of 13, whereas in the female the first positive response was obtained during the 11th year of life. These data corre-

[5] We are indebted to Miss Penelope Hill and Miss Frances Broughton for the creatinine determinations, and to Miss Margaret Amey for painstaking care to insure accurate urinary collections.

late very well with the onset of puberty in both sexes, since, as can be seen from the data presented for the development of secondary sex characteristics, the female matures somewhat earlier than the male. The relationship of the first positive sign of FSH to the menarche in the female is extremely interesting, for FSH could be detected for at least a year before the first menses. This increase in the output of FSH com-

TABLE 1. MALES

Number	Age	FSH (Pos. or Neg.)
1	9· 6	−
48	9· 6	−
44	10-11	−
	13· 3	−
45	11· 1	−
	13· 9	−
47	11· 3	−
14	11· 9	−
29	12· 9	−
37	13· 0	+
2	13· 5	+
32	13· 6	+
16	13· 6	−
40	14· 1	+
33	14· 3	+

TABLE 2. FEMALES

Number	Age	FSH (Pos. or Neg.)	Age Menarche
10	9· 1	−	
18	9· 8	−	
12	10· 0	−	
15	10· 4	−	
	12-11	+	13· 0
6	10· 3	−	
	10· 9	−	
	11· 3	−	
	11· 8	+	12· 7
9	10· 9	−	
	11· 3	+	11· 8
25	11· 6	−	
	11· 9	−	14· 5
51	11· 6	−	
17	11· 9	+	11· 6
	12· 3	+	
52	11· 6	+	12· 5
	13-11	+	
46	12· 0	−	13· 7
5	12· 5	+	13· 3
	12· 9	+	
26	13· 3	+	13· 6
	13· 9	+	
22	14· 8	+	
	14-11	+	14-11

pares favorably with the rise in the estrogenic output which occurs about the same time. It is possible that if daily determinations of FSH were made, a cycle similar to the estrogens may have been evident. This is suggested by the work of Catchpole and Greulich (16), who made repeated studies on 8 female adolescents over a period of time. They found a wide fluctuation in the hormone excretion from day to day in the same subject, as well as widely differing total pictures for the several subjects. Definite

immaturity was associated with absence of the hormone, and the frequency of appearance and amount of the hormone intensified in subjects approaching the menarche. They postulated that pituitary gonadotropic activity is demonstrable for at least a year prior to the menarche. Our data are too few to attempt such a correlation with development, but they agree insofar as the menarche is concerned. These same workers with Sollenberger (17) also studied the excretion of FSH in adolescent males. Of 18 boys aged 11 or under, no hormone could be detected, whereas of 15 boys between 12 and 16 years of age, 12 gave a position response. The amounts recovered were related to developmental status rather than to chronological age. As in the case of the girls, our data agree with theirs.

Several other workers, (18) have reported on the detection of FSH in the urine of adolescents. Frank (5) stated that even children of 4 years of age excreted the hor-

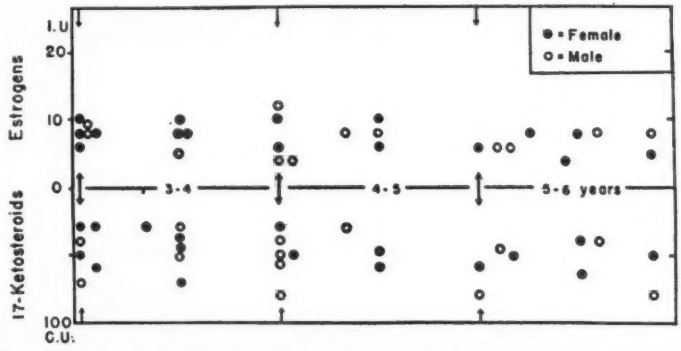

Fig. 1. Scattergraph of 17-ketosteroid and estrogen excretion. Single 24-hour determinations on children of varying ages. The base line is in the center of the charts and 17-ketosteroids are plotted downward from this zero line. They are recorded in color units. Estrogens are recorded from the same base line, and are plotted above the base line. The farther above the base line the higher the titer. The numbers in parentheses are the values too high to be properly placed on the chart. Estrogens are recorded in I.U. equivalent in biological activity to estrone. 17-ketosteroids are recorded in color units where one color unit (C.U.) is equivalent by colorimetric titration to 60γ of crystalline androsterone. The figure over each circle refers to the number of the individual in this study.

mone, at 9 years of age the value was doubled, and just before the menarche was one-half the adult amount. Saethre (19) could not find the gonadotropic hormone in adolescent children between the ages of 11½ and 14, but he did not assay for values below 55 M.U. per liter.

It appears, therefore, that the gonadotropic activity of the anterior pituitary is increased before the onset of puberty, the females showing this change slightly earlier than the males. This evidence of increased activity therefore appears at approximately the time when there is also an increase of excretion of the urinary sex hormones in children as they approach maturity.

17-Ketosteroids and estrogens. Influence of age. By looking at the estrogen and 17-ketosteroid charts, both those which include only individual determinations (fig. 1–4) and those which include consecutive assays (fig. 5–9) it is clear that from 3 years of age to nearly 7 years of age children excrete a fairly constant, though small amount, of both androgens and estrogens. At about 7 years of age the rate of excretion of these substances begins to increase. From the summarizing chart (fig. 10) it is obvious that until nearly 9 years of age, boys and girls excrete analogous amounts of male and female sex hormones, though the ratio of androgen: estrogen is a little higher in boys than girls, with their higher average androgen and lower average estrogen excretion.

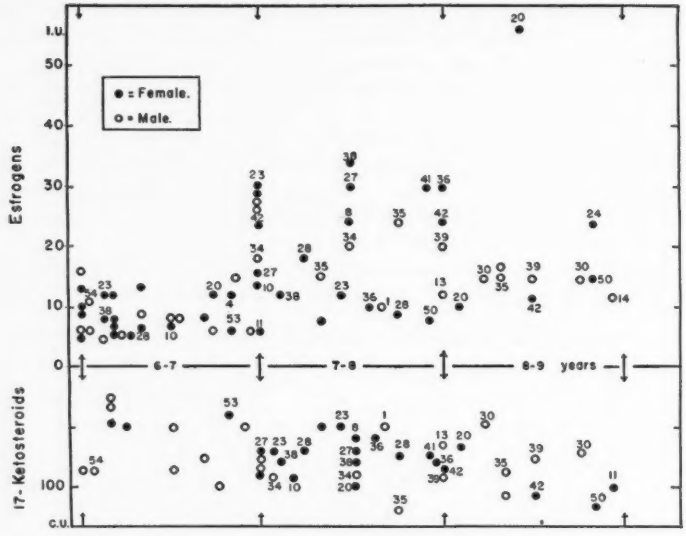

Fig. 2. SCATTERGRAPH OF 17-KETOSTEROID AND ESTROGEN EXCRETION, SINGLE 24-HOUR DETERMINATIONS ON CHILDREN OF VARYING AGES. (See legend of fig. 1 for further details.)

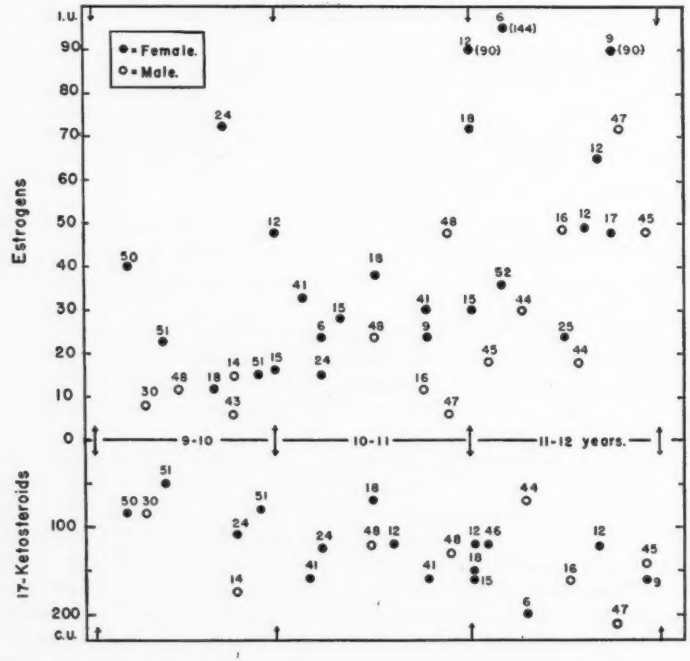

Fig. 3. SCATTERGRAPH OF 17-KETOSTEROID AND ESTROGEN EXCRETION SINGLE 24-HOUR DETERMINATIONS ON CHILDREN OF VARYING AGES. (See legend of fig. 1 for further details.)

From 9 to 11, however, boys tend to increase more rapidly in their androgen excretion, and girls increase more in their estrogen excretion, so that the ratio of androgens to estrogens definitely changes in the two sexes, rising in the boys, declining in the girls. After 9 years of age greater scattering begins to occur, as excretion of both male and female hormones becomes progressively greater (fig. 3, 4). These changes do not always occur at the same chronological age, inasmuch as the rate of development naturally varies in different children. These levels of sex hormone excretion correlate far better with physical development than with actual age. At about ten or eleven years of age cycles of excretion of estrogens become obvious in the girls, so that the

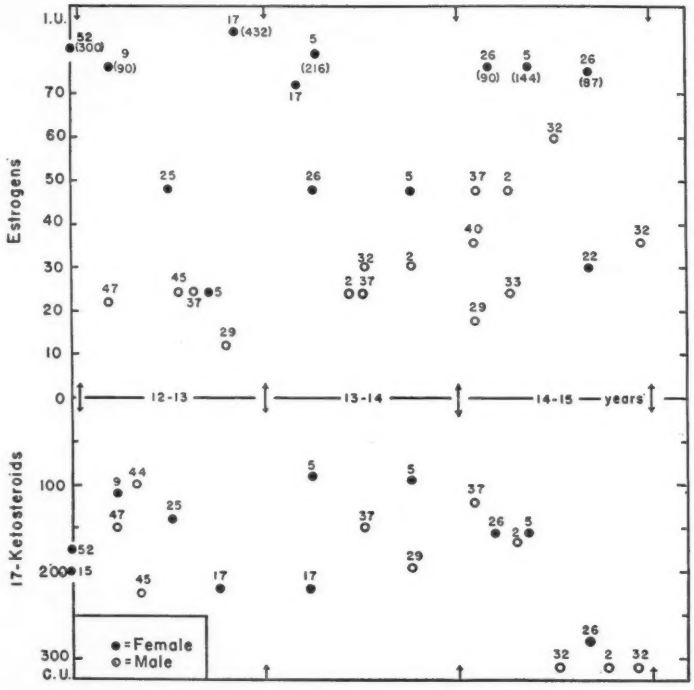

Fig. 4. SCATTERGRAPH OF 17-KETOSTEROID AND ESTROGEN EXCRETION SINGLE 24-HOUR DETERMINATIONS ON CHILDREN OF VARYING AGES. (See legend of fig. 1 for further details.)

averaging of these determinations in this series (fig. 10) gives an index of monthly excretion rates, but a fallacious idea of daily excretion levels, for the level in girls is raised out of all proportion to their minimal rates. But if one takes the lower excretion rates of girls, one still finds that they become markedly elevated after 11 years of age, a change which appears to be progressive up to the menarche. This elevation is in contrast to the estrogen excretion in boys which rises only a little during this same period.

Sex difference. Striking differences in sex hormone excretion rates become obvious only at about the age of 9 or 10 years in girls and boys (fig. 10). Before that the difference is not great. As puberty approaches, very obvious variations in the excretion rate appear, particularly in relation to the estrogen component. The importance of this change can be better seen in the curves which show consecutive determina-

tions, for the charts demonstrate the dramatic changes in estrogen excretion rates in girls with the appearance of a definite cycle of excretion at least one and a half years before the onset of the menarche (fig. 7–9). The levels of excretion in the cycle become progressively greater until the menarche appears. In *case 15* (fig. 8) we were fortunate enough to have assayed a series of specimens which ended just 8 days before the menarche, and so we obtained a further series from the 10th to the 22nd day after the beginning of this first catamenia. The observations demonstrated a definite cycle long before the menarche, and a level of excretion just before the first menses, analo-

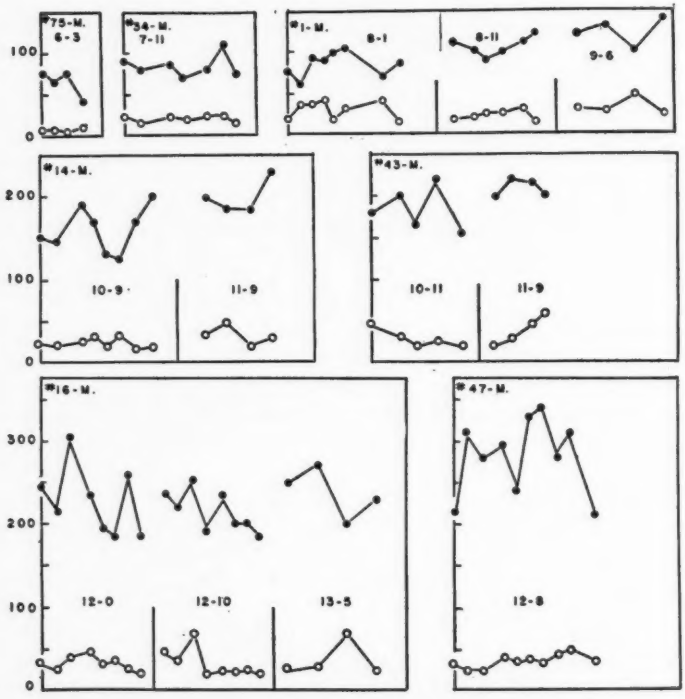

Fig. 5. Consecutive determinations of 17-ketosteroid and estrogen excretion in individual boys. Each case is blocked in, with sex and number in left upper corner, and age in the middle of the chart. The 24-hour urine samples were collected twice a week. The vertical lines half way up the chart represent time intervals between cycles.

gous to that which subsequently followed it. Such cycles of excretion appear only in girls, and have also appeared only in the estrogenic hormones. Boys do not show this cyclic variation and their estrogen excretion increases only at a linear rate, which, however, is still slightly below the minimal excretion level of girls of the same age, (fig. 5–6).

The 17-ketosteroid excretion, on the contrary, shows no cyclic variation in either sex. It increases in rate fairly rapidly in both sexes after the age of nine, but more so in boys than in girls. The fact that both 17-ketosteroids and estrogens rise in both sexes, long before a cycle of estrogen appears in girls, indicates that the primary stimulation of the peripheral endocrine glands results in a constant secretion, and the cyclic secre-

tion is superimposed on this initial change. In boys the physical changes of puberty accompany the simple continued elevation of androgen excretion. These data indicate the necessity of repeated determinations of estrogens in girls if one is interested in the approach of the menarche, but they also indicate that such elaborate observations are not necessary in boys who do not have dramatic fluctuations.

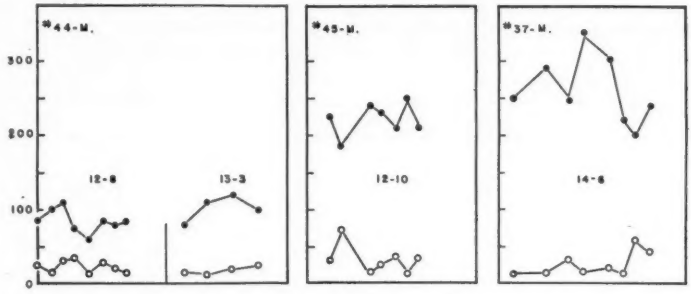

Fig 6. CONSECUTIVE DETERMINATIONS OF 17-KETOSTEROID AND ESTROGEN EXCRETION IN INDIVIDUAL BOYS. (See fig. 5 for further details.)

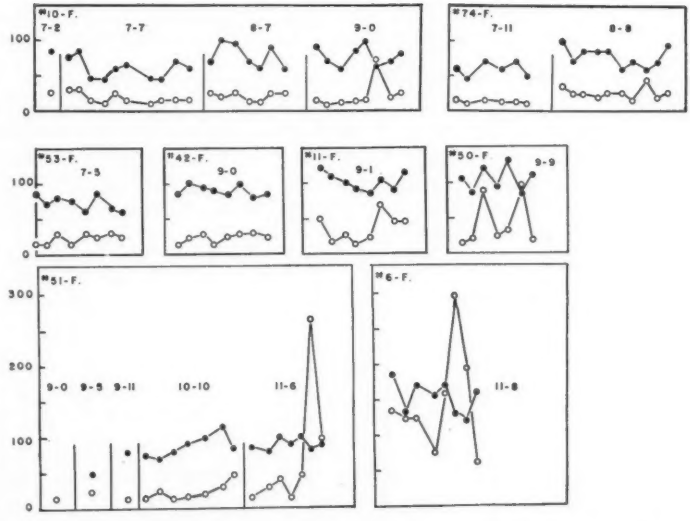

Fig. 7. CONSECUTIVE DETERMINATION OF 17-KETOSTEROID AND ESTROGEN EXCRETION IN INDIVIDUAL GIRLS. The black rectangles at bottom of charts are days of menstruation.

In girls it is clear that the cyclic excretion of estrogens precedes the appearance of striking secondary sex characteristics. The change from the neuter configuration of younger children, therefore, probably follows this shift in excretion. This can be seen in an instructive cycle obtained from a girl (Pt., fig. 8). At 14 years and 8 months there was no abnormality except that there was great retardation of growth and no evidence whatsoever of secondary sex changes. A series of urinary assays were obtained and they showed a definite large variation in estrogen excretion rates. The 17-ketosteroids also showed evidence of increased excretion. She was then given intermittent

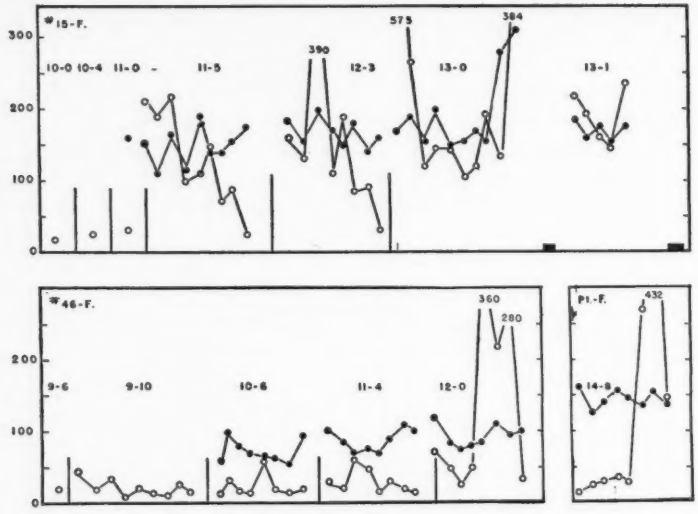

Fig. 8. CONSECUTIVE DETERMINATION OF 17-KETOSTEROID AND ESTROGEN EXCRETION IN INDIVIDUAL GIRLS. The black rectangles at bottom of charts are days of menstruation.

injections of a growth hormone which contained no gonadotropic factor. Three months after this series of determinations was completed, a definite breast development became obvious, followed by a rapid development of secondary sex characteristics. The menarche appeared just one year later and great growth acceleration also occurred during this time for during this year she grew 3¼ in. to a height of 59", and gained 24 lb., to a total of 104 lb. The menarche of *case 6* occurred eleven months after the last hormone determination.

Another example is shown by *case 25*. This girl at 13 years and 6 months had a

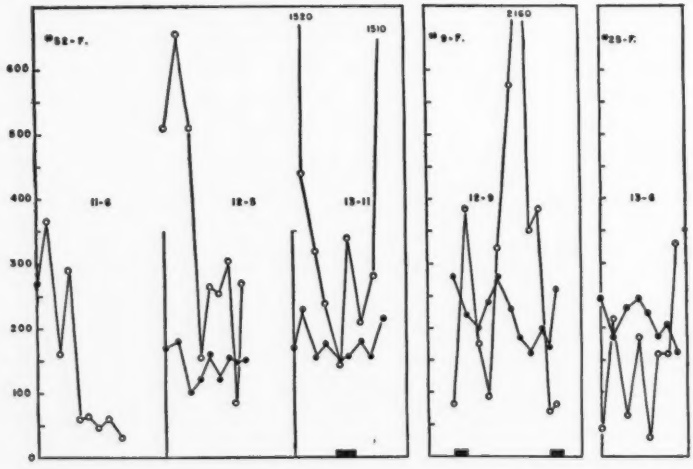

Fig. 9. CONSECUTIVE DETERMINATION OF 17-KETOSTEROID AND ESTROGEN EXCRETION IN INDIVIDUAL GIRLS. The black rectangles at bottom of charts are days of menstruation.

much lower estrogen excretion than other girls of her age, with only a suggestion of a cycle in the last observation. It was just this time that she began to show secondary sex characteristics, and menstruated first at 14 years and 5 months. This child also showed a retarded bone age, creatinine excretion below normal, and body size which was below the average normal. She showed a spurt in growth before catamenia started.

The studies on *case 46* are very interesting. Up to the age of 11 years and 4 months her estrogen excretions were very low, and androgen excretions were also lower than in the other girls. At 12 years of age the androgens were still low and many of the

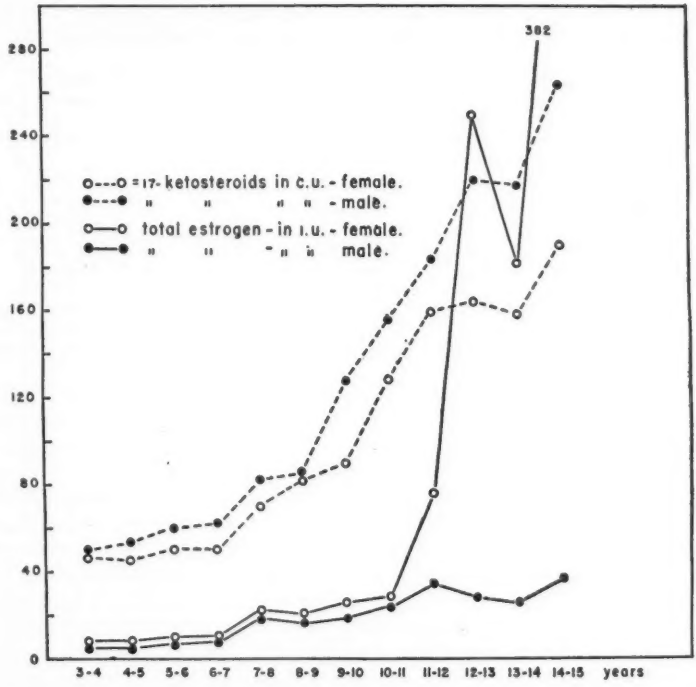

Fig. 10. Summarizing chart of all determinations of excretion of 17-ketosteroids and estrogens in girls and boys of different ages. Consecutive assays are averaged and used as a single determination.

estrogen determinations were far below normal, although she then demonstrated for the first time a cyclic excretion with relatively high levels. At this same age she showed no secondary sex characteristics, her bone age was distinctly retarded, creatinine excretion was below the normal, and her body measurements were somewhat below standard. Eight months later she demonstrated the beginning of pelvic hair and very early breast changes, and the menarche appeared 18 months after the last hormone determination.

In boys also the general level of 17-ketosteroid and estrogen excretion appears to be important. A good example of the relationship of sex hormone excretion to growth and puberty is seen in *case 44*. The chart demonstrates the obviously low excretion rates obtained in his urine, as compared with all the other examples of similar age, for at 13 years and 4 months he had essentially a 9-year-old excretion rate.

At that time he was very undersized and had not yet begun to show the physical changes characteristic of early puberty, although he showed a normal bone age. Six months after this cycle he still showed no evidence of puberty except for a few silky short pubic hairs. In this 6 months he had grown only 1 in. and gained 6 lb. This boy is, therefore, very much retarded not only in his hormone excretion rate but also in his whole growth and maturation rate.

What is the source of these hormones? It is probable that only a small quantity comes from the testes and ovaries in the younger children, but it may well be that a fair amount of them are derived from the secretions of the adrenal cortex. How much of the early increase in these hormones comes from this alone is not now known, but the mere fact that girls and boys have an analogous concentration of 17-ketosteroids and estrogens, suggests that a fairly high percentage of these hormones may be de-

TABLE 3. AVERAGE WEIGHTS OF ENDOCRINE ORGANS AT VARIOUS AGE LEVELS

Age	Hypophysis	Thyroid		Adrenals		Testes	Prostate	Ovaries[1]	Uterus
		M.	F.	M.	F.				
	cgm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1- 5	26.6	6.1	4.4	4.6	4.2	2.9	0.8	1.0	1.8
6-10	38.5	8.4	7.8	6.6	6.0	2.9 (6–12)	1.9	1.9	2.3
11-15	50.0	12.5	11.5	8.6	8.0	20.6 (13–17)	8.2	4.0	8.0
16-20	69.5	23.5	20.7	13.0	11.0	29.2	8.2	8.3	27.0

[1] All data taken from Roessle and Roulet (24) except on ovaries which are quoted by Gruelich.

rived from the adrenal cortex. This is the most likely source inasmuch as its peculiar hormone, corticosterone, is chemically so closely related, and inasmuch as androgens have also been isolated from this gland (20). Further evidence for this, recently summarized by Wilkins, Fleischmann and Howard (21), is found in cortical tumors which produce an excretion of enormous amounts of both androgens and estrogens. This is even true when tumors are found in children of very early age (22). Other evidence can be found in the excretion of these substances in female castrates, as well as after the menopause (23) when the ovaries are no longer functioning.

The rate of weight gain of endocrine glands also lends suggestive evidence. Table 3 shows this in human tissues compiled from figures derived from Roessle and Roulet (24) and from Wehefritz (25). The table makes one observation clear: childhood is associated with a gradual growth of hypophysis, thyroid and adrenal glands, but there is relatively little growth of the sex organs until several years before maturation. This may be due to differing degrees of pituitary stimulation, or to relative receptivity of the different peripheral organs. We have no adequate data in regard to this, except that the gonadotropic hormones of the pituitary were not obvious by our technic until 11 years of age in girls and almost 13 years of age in boys. Probably these pituitary hormones are present before this time, but they obviously increase rather suddenly when the weights of the primary sex organs and the rate of sex hormone excretion also abruptly rise. In early childhood and adolescence the curve of weight increment of the adrenals is fairly analogous to the composite curves of the 17-ketosteroids and estrogen excretion rate in both boys and girls. At 11 or 12 a new mechanism appears which may be attributed to an increase in gonadal activity, since in the male the rise in the excretion of the 17-ketosteroids corresponds in time to a significant increase in testicular weight, and in the female the rise in estrogen to a rapid gain in ovarian weight. The development of these increases and also of secondary sex charac-

teristics appear to be primarily dependent upon gonad secretions (table 4). This divergence of these excretions after the age period becomes more marked as the gonads increase in weight and the children approach the adult state. However, the fact that the 17-ketosteroids in the female and the estrogens in the male continue to parallel the constant slow gain in adrenal weight, as the children grow older suggests that a fraction of the sex hormones still may come from this organ.

Continuing this train of thought further, the prostate and the uterus may be considered secondary sex organs, probably largely stimulated by the gonadal activity. Moore (26, 27) states that there is no essential change in the prostate from the first

TABLE 4. AVERAGE TIME OF SECONDARY SEX CHARACTERS IN BOTH SEXES[1]

SEX CHARACTER	ORDER OF DEVELOPMENT
	Females
1. Growth of pelvis	Precedes development of breasts
2. Breast	10-11 years—first real change
3. Vaginal secretion	Follows growth of breasts. Changes from pH 7.2–8.0 to 4.3 with cornification and deposition of glycogen
4. Pubic hair	These usually follow the above in the order given
5. Axillary hair	
6. Menarche	
	Males
1. Penis and testes	Precedes first appearance of definitive pubic hair
2. Prostate	Shows activity at about 10–11 years of age
3. Pubic hair	Follow above in order named
4. Axillary hair	
5. Facial hair	
6. Voice change	15 years average age
7. Spermatazoa	12–15 years of age

[1] From Gruelich, et al. (28).

to the tenth year. At this time there are changes suggestive of subtotal physiological stimulation which may result from an increased secretion of the testicular hormone. This stimulation continues to puberty when there is a rapid maturation of the prostate. As regards the uterus, there is a fairly rapid increase in weight after the eleventh year. Hence, it can be inferred that the menarche is a manifestation of sufficient uterine development to allow menstruation, as well as a sudden change in the hormonal secretions of the female. Development of both the prostate and uterus therefore parallel or closely follow upon the gonadal weight and the changes in the excretion of sex hormones.

DISCUSSION

From the data reported in this paper one can arrive at a fairly clear understanding of the physical mechanisms involved in the sexual development of children.

The determinations of the follicle stimulating hormone of the pituitary gland are the least satisfactory of the analyses reported. It is probable that if the technic were better one could detect this hormone earlier in life. However, it is obvious from our data that several years before sexual maturity one can obtain a positive test for this hormone in the urine, whereas before this time it is not present in sufficient quantity to discover it by our technic. It seems clear, nevertheless, that at this age period there is an increase in the secretion of this anterior pituitary hormone which corresponds to the time when dramatic increases occur in the excretion of the sex hormones from the peripheral glands. It is probable, though we have not proven it, that this increase in anterior pituitary hormone precedes the others.

Sex hormones in childhood. In early childhood, namely, from about 3 to 7 years of age, both 17-ketosteroids and estrogens have been found in all urines. They are pres-

ent in small quantities and the interesting thing is that they exist in approximately the same amount in both boys and girls. It is not possible to determine the sex of a child at this age by the excretion of these hormones. This is also the period when the general build of both boys and girls is not strikingly different. We have already discussed the possible origin of these hormones and have presented evidence that some of them may come from the adrenal cortex rather than from the ovaries and testes. Indeed, it is very likely that the adrenal continues to secrete some of these hormones in adult life. However, it is also possible that the ovaries and testes are factors in the formation of these small quantities in young children because, in animals and in human beings ovariectomy at an early age results in smaller uteri. Therefore, there is at least some relationship between the ovary and the size of the uterus, which suggests that the ovary is secreting stimulating estrogens even in early life. Further evidence in regard to this problem in man is being accumulated in this laboratory.

At about 8 years of age the excretion of 17-ketosteroids and estrogens begins to increase. Between 8 to 11 years, this increase is gradual and the excretion of both components, except for minor fluctuations of the estrogens, is fairly constant from day to day. During this period there is a more dramatic elevation of the 17-ketosteroid component than there is of the estrogen, and this rises more rapidly in boys than in girls. What part of this comes from the adrenals and what part from the testes is not perfectly clear but in the weight charts of these various organs it is seen that the weight of the testes increases very slightly in this period whereas the weight of the adrenals continues to increase constantly. At approximately 11 years of age a suddenly increased excretion occurs which coincides with a sudden acceleration in growth of the testes. At this period the excretion of the 17-ketosteroids increases more markedly in boys than in girls, which suggests that a fairly high percentage of this additional excretion is of testicular origin. It is also at this time that the first signs of the secondary sex characteristics are apparent in the male. In girls, there is a continued increase in 17-ketosteroids excretion which is more gradual but whether this continues to come from the gradually growing adrenal, although it seems likely, cannot now be established.

Estrogen excretion in boys continues at a fairly low level and has no dramatic increase as puberty develops. In girls there is an increase in the excretion rate which appears at about eight or nine years of age, and then becomes enormously accelerated after the age of eleven. In the beginning of this increase there are slight fluctuations from day to day but without cyclic manifestations. It appears likely, however, that it is at this time that one begins to see the differential development of the female pelvis and other generalized sex changes such as is obvious in the musculature and general configuration of the body. Then a new mechanism appears in the development of the adult cycle of estrogen excretion. The evidence which we have been able to accumulate indicates that this precedes by a little the onset of the striking secondary sex changes which occur at puberty. In a few cases which we were fortunate enough to observe, the appearance of a cycle preceded the development of the breasts and hirsutism. These first evidences of a cycle preceded the menarche by approximately a year and a half, and it is probably during this period that the accessory organs, like the uterus, rapidly develop. The initiation of these characteristics seems closely linked to the striking changes which occur at this time in the sex hormone excretion as well as to the growth of both ovaries and testes. Dr. Sidney Farber, pathologist at the Children's Hospital, tells us that one can frequently find evidence of ovulation in early childhood, certainly from the 8th year, yet it is about 1½ years after the onset of a cyclic excretion of the follicular hormone before menstruation is established. Therefore, it would appear likely that the onset of menstruation is not only dependent

upon the ovarian secretion but also upon subsequent, adequate development of the uterus.

SUMMARY

From three to seven years of age, both boys and girls excrete a small and constant amount of estrogens and 17-ketosteroids in the urine. There is very little difference between the sexes.

From eight to eleven years of age there is an increased excretion of these sex hormones; 17-ketosteroids increase more rapidly in boys, estrogen in girls.

About one and one-half years before the menarche, estrogen excretion becomes cyclic in girls; and the intensity of these cycles gradually increases. The appearance of estrogen cycles precedes the dramatic sexual development of girls before the menarche. No cycles are obvious in the excretions of boys.

There is no cycle in 17-ketosteroid excretion in either sex. Boys, however, have a higher excretion, especially after eleven years of age when secondary sex characteristics begin to appear.

At eleven years of age the follicle stimulating hormone of the anterior pituitary gland in the urine of girls was first detected, but in boys it appeared somewhere between the twelfth and thirteenth year. This late appearance may be due to the inefficiency of the method.

It appears clear, therefore, that the menarche in girls and the sexual maturity of boys are the culmination of an endocrine development which starts about 5 years before puberty. This is gradual until approximately 1½ years preceding maturity, when an additional mechanism appears which leads to more rapid differentiation of sex characteristics and eventually complete metamorphosis.

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THE CLOSE CORRELATION OF ANDROGEN AND CREATININE EXCRETION IN NORMAL CHILDREN[1]

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IN A PREVIOUS STUDY (1, 1a), the urinary excretions of the 17-ketosteroids (some of which are androgenic) and estrogens in boys and girls were determined. It was shown that the 17-ketosteroids increase gradually with advancing years in both sexes; boys having a somewhat higher level than girls particularly after 11 years of age. However, the difference in the levels between the sexes was not very great. The estrogens also increase gradually with age, with a higher level in girls, especially after 10 or 11 years of age, when a marked distinction between the sexes develops, due to the appearance of a cyclic excretion in the female. The 17-ketosteroids, as far as can be ascertained, do not have cyclic changes in excretion rates, but remain at a fairly constant level at any given age period in both sexes. In order to determine the accuracy of the collection of the 24-hour urine specimens in these cases, analyses of creatinine were also made. It seems reasonable, therefore, to attempt a correlation of the creatinine excretion with that of the urinary sex hormones. This was possible with the 17-ketosteroids because of the fairly uniform excretion from day to day. It was not considered feasible in the case of the estrogens, because of the great daily variation which appears in girls as the menarche approaches and also because of the widely divergent levels between the two sexes which commences at about 9 years of age. It is possible that there may be a correlation of creatinine with estrogens within each sex, since both estrogens and creatinine increase gradually with age, but we have not considered this because of the complexity of the problem.

Technics. The 17-ketosteroid determinations were those described in the preceding paper (1). Preformed creatinine was determined on these 24-hour urinary collections by the method described by Folin (2) except that the readings were made with a photo-electric colorimeter.

RESULTS

A total of 396 creatinine determinations were made on 36 boys and 39 girls. In those instances where consecutive semi-weekly assays were made the average value was used. Many of the data represent observations on the same children every 6 months over a period varying from 2 to 4 years. The children ranged in age from 3 to 14 years.

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Figure 1 represents the 17-ketosteroids, of which the androgens are a part, plotted against the creatinine excretion. This was done without regard to age. Correlation of this material with chronological age, sexual maturity, physical development, weight, and other relevant data will be presented in another publication (3). The correlation of the data shown in figure 1 is given in table 1.

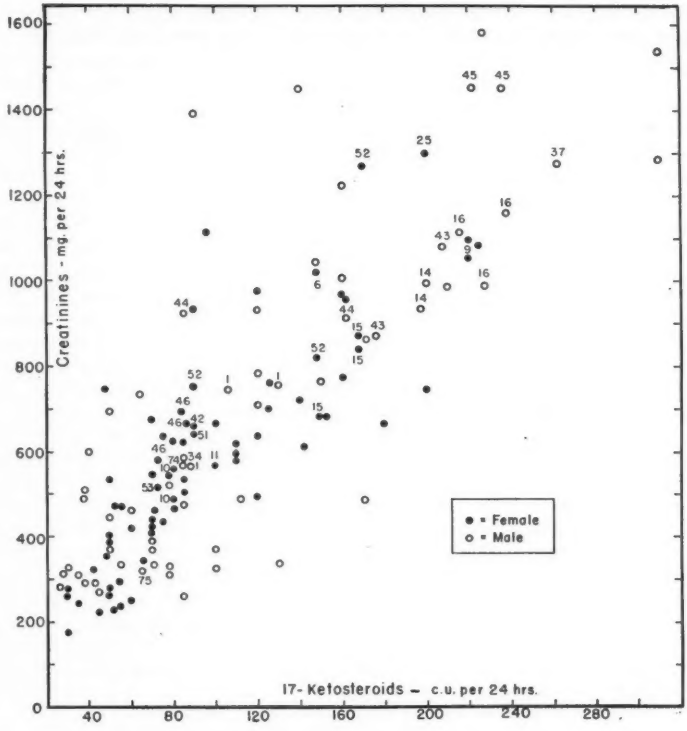

Fig. 1. Excretion of 17-ketosteroids and creatinine in 24-hour urine samples, obtained from children varying in age from 3 to 14 years.

Table 1

	Mean creatinine	σ	Mean 17-keto-steroids	σ	Coefficient of correlation	No. of observations
Males	716	383	122.1	73.6	+0.82	61
Females	601	257	100.7	52.4	+0.79	74

Creatinine expressed in mg. per 24-hour specimen; 17-ketosteroids expressed in color units (c.u.); where one c.u. is equivalent to 60γ of crystalline androsterone.

These figures imply a very high coefficient of correlation which is obviously significant.

Literature. The literature largely deals with the relation of the sex hormones to creatine excretion, while the creatinine metabolism has been more or less ignored. Fascinated by the

irregular appearance of creatine in various sex abnormalities, the literature focuses on this relationship. This interest harks back to the early discovery of its irregular excretion in women, particularly during pregnancy and immediately after the puerperium. Creatine has been found in the testes of bulls (4), dogs (5), and sheep (4) in concentrations of about one-half that found in striated muscle while in other parenchymatous organs like liver (4), pancreas, kidney, spleen, thyroid and thymus (6, 7) it is present in only very small amounts. In 1921 Read (8) found creatine was constantly present in the urine of eunuchs who had been castrated before the development of secondary sex characteristics, with what appears to be extraordinarily low creatinine excretion. In 1922 McNeal (9) reported creatine in the urine of 6 adult males, several of whom had been castrated in adult life. The creatinine excretion appears adequate, though the necessary data for measuring muscle mass is not given. Beumer and Fasold (10) found that very young children, who reach puberty prematurely are able to dispose of exogenous creatine as completely as do adults, and Fasold (11) finds that the administration of large doses of the ovarian hormone is without influence upon the creatine metabolism of normal children. Buadze (12) thought it probable that the male sex hormone inhibits the output of creatine, and that the female hormone promotes its appearance in the urine. Nitzescu and Gontzea (13) arrived at approximately similar conclusions. Schlittenhelm and Buhler (14) studied the creatine-creatinine metabolism in internal secretion disorders and showed that the sex glands had a large influence on creatine excretion; it was inhibited in normals, but excreted in abnormal function. The use of a testosterone preparation (proviron) made this creatine excretion disappear in sex deficient males for a day or two, but the estrogenic substance, alpha estradiol (progynon) had no such effect (15). This effect was not observed in most women at the menopause. The creatinine excretion varied considerably from day to day and the data are not given to enable one to judge the amount in relation to body build. Beard and his coworkers (16) found that estrone (theelin), testosterone propionate (perandren) and A-P extracts caused increases in muscle creatine and in creatine excretion, accompanied by decreases in creatinine excretion. They found that removal of ovaries and uterus, but not the testes, caused significant increases in muscle creatine. Kenyon et al., (17) gave testosterone propionate in very large doses and found the excretion of creatinine was unaffected. Intensified creatine excretion, by adding it to the diet of a eunuchoid, was reduced by the giving of androgen and increased during recovery. Muschat, Labess and Meranza (18) used a creatinine utilization test and concluded it was an indicator of the amount of gonadal activity in the male inasmuch as the eunuch showed a greater retention than normals. Schrire (19) studied creatine-creatinine metabolism in acromegaly and found both components in abnormally high and surprisingly irregular quantities in the urine. He concludes that there are two factors in the pituitary gland one which elevates creatine in the urine possibly via the thyroid and the other controls creatinine, but both are independent in their action.

DISCUSSION

From the literature we find that very little attention has been paid to actual creatinine excretion, but that much has been focused upon the phenomenon of creatine excretion. Moreover, unanimity is not found in this field. The literature indicates that both girls and immature boys have a distinct creatine excretion, an excretion which continues irregularly in women after they mature, but which tends to disappear in males. It is true that more recently Light and Warren (20) found creatine in a large percentage of boys fourteen to nineteen years of age, and Taylor and Chew (21) found it in young adult males in varying amounts but these are not the usual reports in the literature, for normal males are usually assumed to excrete no creatine, and this is a finding which has been corroborated by Rawson (22) in this laboratory. However, there is evidence from the literature that the injection of testosterone causes a temporary disappearance of creatine in the eunuchoid male, and that in acromegaly both creatinine and creatine appear in abnormally large quantities, but surprisingly enough no attention has been paid to the relationship of excretion of creatinine and the 17-ketosteroids. This is largely explained by the fact that analyses

for 17-ketosteroids have only recently become available. Harding and Gaebler (23) studied children between the ages of 2½ and 9½ and found that although creatinine increased with age no difference could be found between the sexes. This is analogous to our findings in regard to the 17-ketosteroids. It is after this age period that the excretion of creatinine as well as that of the 17-ketosteroids begins to show a difference in the two sexes. This can be seen in the scattergrams of our previous paper (1). These and our observations above indicate that there must be some definite relationship of these apparently unrelated chemicals. Several possibilities naturally occur to explain this phenomenon, though the final explanation cannot now be made.

Creatinine excretion is generally accepted as an indication of muscle mass. It is interesting in this regard that Hodgson and Lewis (24) found creatinine coefficients of the same order of magnitude as those of men in a series of women with physical development greater than those of ordinary women. Furthermore, they found a frequency of creatine excretion similar to that which is ordinarily observed in women, indicating that creatinuria in women is probably not related to the difference in muscular development between men and women. The increased muscle mass in boys in comparison with girls has never been explained, but it might be partially dependent upon the rate of androgen activity. Papanicolaou (25) found that he could increase the muscle mass in young guinea pigs by the injection of testosterone. If this be so, an increased formation of androgen might cause an increased formation of muscle so that there would be a high correlation between the two and hence with creatinine. Clinically this appears to be so; the sexually well developed boys and men with high androgen excretion are also well muscled. Inasmuch as we are in the process of determining the 17-ketosteroid excretion in muscular women, we cannot say yet what role it plays in this group. The creatinine content of the urine does not affect the determination of 17-ketosteroids, and it seems highly unlikely that muscle mass, in itself, should influence the determinations.

CONCLUSIONS

In growing children, both girls and boys, there is a close correlation between the excretion of the 17-ketosteroids and of creatinine. The correlation of these two substances is so close that it seems highly likely that there is a fundamental relationship between them. The most likely explanation appears to be that androgens have an influence on the muscle mass of the individual so that an increase of androgen formation would result in an increased muscle mass, and therefore the creatinine excretion rate.

The observations are being repeated on young college students, where the factors of age and growth will be eliminated.

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ANDROGEN PRODUCTION IN THE FEMALE RAT

THE OVARY AND THE ADRENAL IN THE IMMATURE RAT[1]

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THE FEMALE PROSTATE, homologue of the male ventral prostate, is present in some female rats. The incidence varies in different strains and may be increased by inbreeding (1). Price (2) demonstrated that during a limited period in the immature female rat (21 to 40 days of age) these prostates exhibit evidence of androgenic stimulation. In a previous report (3) we confirmed this finding. Included in this report were a few cases of castrate immature females which also had prostates showing some androgenic stimulation. This finding was indicative evidence for the hypothesis advanced by Price, that the female adrenal cortex might have a temporary andromimetic function such as has been demonstrated by us in the immature male rat (4, 5).

The present report concerns further investigation on this matter, specifically the respective roles of the immature ovary and adrenal in the production of the androgenic substances responsible for stimulation of the immature female prostate.

PROCEDURE

A strain of rats having a high incidence of female prostates was used.[2] The ventral prostates of normal, castrate, adrenalectomized and castrate-adrenalectomized females were compared as to gross weight and histological findings. Castration was performed at 16 days and adrenalectomy at 21 days of age. All animals were killed at 26 days of age. This is the same procedure used in the previous studies on the immature male adrenal. As before, all animals were left with the mother until killed. Bread was given as dietary supplement and 1% NaCl solution was substituted for drinking water.

Sixty animals were used in this study, but only 41 provided data. Two animals died following adrenalectomy, 3 were identified as "slips," that is, containing extraneous adrenal tissue, and 14 had no prostates. Since the female prostate is sometimes unilateral and sometimes bilateral the data for each group are given separately for each of these two conditions. By a rare coincidence the same number of single lobes and double lobes were found in each group. Because of the small number of cases in each of the sub-groups the data was subjected to rigorous statistical analysis. Differences between groups were not accpeted as valid unless the critical ratio was 3.00 or more. Table 1 shows the complete data.

RESULTS

Compared to the normal, the castrates had a somewhat smaller mean prostatic weight. With the single lobe prostates this difference was not significant (critical ratio,

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[2] Dr. E. Witschi kindly provided the founders of this strain of rats.

1.17), whereas with double lobes, the castrate prostates were significantly smaller than the normal (critical ratio, 3.65). Both single and double lobe prostates of the adrenalectomized castrates were significantly smaller than those of the castrates (respective critical ratios, 3.56 and 3.41). Removal of the ovaries, therefore, caused a decrease in prostatic weight which in turn was greatly exaggerated by removal of the adrenals as well. These findings demonstrate that the immature ovary produces sufficient androgen to stimulate the prostate. They also demonstrate that, in the absence of the ovary, the adrenal also can produce sufficient androgen to prevent complete atrophy.

The prostates of the animals which were only adrenalectomized did not differ significantly from the normals (critical ratio, 0.61 for single lobe prostates; 0.78 for

TABLE 1

Group	m. body wt.	No. animals	m. prost. wt.		Critical ratios		
			Single lobe	Double lobe	Cast.	Ad.	Ad.-Cast.
	gm.		mg.	mg.			
Normal	35.5	5	3.76 ±0.97*		1.17	0.61	3.30
		5		10.50 ±1.48	3.65	0.78	5.80
Castrate	34.1	5	2.46 ±0.51				3.56
		5		4.48 ±0.72			3.41
Adrenalectomized	35.0	5	5.22 ±2.17				
		6		11.96 ±1.16			
Adrenalectomized castrates	33.5	5	0.46 ±0.22				
		5		1.51 ±0.48			

* $\sigma_m = \frac{\Sigma d^2}{N(N-1)}$ C. R. $= \frac{\text{obtained difference}}{\sqrt{(\sigma_{m_1})^2 + (\sigma_{m_2})^2}}$

double lobes). This fact eliminates the possibility that the regression of the castrate prostates after adrenalectomy might be an expression of general debility due to lack of cortical hormone. Since the prostates of the adrenalectomized controls were on a level with the normals, the general condition of the animals could not be considered to have affected the prostates. Actually, all adrenalectomized animals seemed in excellent condition when killed.

The histological findings corroborated the gross weight findings. Eight prostates from adrenalectomized castrates were examined. All were atrophic in appearance. The acini were uniformly small with a low epithelium which showed no clear areas; the stroma of the gland appeared to be increased in amount and was more dense than in the normal. Seven prostates from castrates were also examined. These prostates presented varied conditions ranging from an almost atrophic level to a nearly normal ap-

pearance. Clear areas were found in 4 of these 7 prostates, but they were somewhat less distinct than in the normal gland. This latter condition probably indicates a somewhat diminished androgenic stimulation compared with the normal. A slightly subnormal degree of stimulation in the castrate prostates would be expected on the basis of the weight findings.

DISCUSSION AND SUMMARY

The prostates of 26-day-old female rats show evidence of androgenic stimulation. After 10 days castration the prostates of the same age females also show evidence of androgenic stimulation, though to a lesser degree. The partial maintenance of the prostates after castration is considered to be due to the formation of androgenic substance by the adrenal, since complete atrophy of the prostates follows removal of both ovaries and adrenals. Removal of the adrenals alone does not produce an effect on the prostates. This finding may be interpreted in two ways. (*a*) The adrenal of the normal immature female does not produce an appreciable amount of androgenic substance, but only assumes this function after castration. In this case the ovary must be considered the chief normal source of the androgen. (*b*) The adrenal does contribute to androgen production in the normal female and, after its removal, ovarian androgen production is increased to compensate for the loss of the adrenal androgen.

CONCLUSION

It is concluded that, in the immature female rat, at least at 26 days of age, both the ovary and arenal are capable of producing physiologically demonstrable quantities of an androgenic substance.

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THE LIVER AND THE ADRENAL ANDROGEN OF THE RAT[1]

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IN PREVIOUS PAPERS (1, 2) data have been presented demonstrating the andromimetic function of the castrate immature rat adrenal. Considerable evidence from other sources (3–7) indicates that the adrenals of the rat, mouse and man, under certain circumstances, produce androgenic substances. Several crystalline substances having androgenic potency have been isolated from bovine adrenals (8–10). In view of these findings it is of interest to know something concerning the nature of the androgen produced by the rat adrenal. Is it identical with the testicular androgen?

Since chemical isolation is not practicable, an indirect approach to the problem has been used. The role of the liver in the metabolism of certain steroid hormones has been extensively studied. Evidence from numerous sources indicates that the liver inactivates estrogens both in vivo and in vitro (11–16). Androgens also have been shown to be inactivated by the liver (14, 17, 18). The present authors have offered evidence (19) that the liver inactivates the testicular androgen of the rat in vivo. The present investigation was undertaken to determine if the adrenal androgen is also inactivated by the liver.

In one of the earlier studies (2) it was found that the andromimetic capacity of the immature male rat adrenal declines with increasing age and is lost entirely at an early age. For this reason, young immature male rats were used in this study.

METHODS

Seventy-five immature male rats were divided into three groups. At 16 days of age all were castrated and at the same time one adrenal was removed from each. In the experimental group this adrenal was enucleated and the capsule sutured to the posterior surface of the stomach. From previous studies it was known that this situation is ideal for grafted tissue when it is desired to have the venous drainage from the graft pass directly through the portal system into the liver. Adhesions with vascular drainage to the peripheral blood system are not encountered when tissues are implanted in this location. In the second group, implant controls, the capsule of the removed adrenal was implanted between the kidney and the body wall or beneath the kidney capsule. This location is favorable for establishment of venous drainage from the implant into the peripheral circulation. In the third group, adrenalectomized castrate controls, the adrenal was not implanted, but appropriate incisions and manipulations of organs were made to provide an amount of trauma commensurable with that produced in the other groups.

On the 5th day after the first operation, at 21 days of age, the remaining adrenal was removed from each of the animals. Thus the animals of the experimental and implant control groups were left with only implanted adrenal tissue, while the animals of the control group were without adrenal tissue of any kind. All animals were

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left with the mother and were given a standard diet of rat pellets supplemented by bread and 1% NaCl solution in place of drinking water.

On the 6th day after the second operation, at 27 days of age, the animals were killed and examined under a binocular dissecting microscope. The location and condition of the implanted adrenal tissue were carefully noted. The implants and ventral prostates were removed and fixed in Bouin's solution. After 24 hours fixation the prostates were weighed on a Roller-Smith milligram balance. At autopsy careful search was made for adrenal remnants or hypertrophied accessory adrenal tissue. All animals having adrenal tissue other than that purposely implanted were not included in the data. Postoperative mortality, the presence of extraneous adrenal tissue and absence of viable grafted tissue reduced the number of animals providing data to 61.

RESULTS

The recovered implants, in most cases, were healthy in appearance and were approximately the size of single normal adrenals at this age. Histological examination showed good regeneration of cortical tissue from the implanted capsule.

TABLE 1

Group	No. animals	Body weight		C. R.		Prost. wt.		C. R.		Pr./B.W.-Ratio		C. R.	
				Impl. cont.	Cont.			Impl. cont.	Cont.			Impl. cont.	Cont.
		gm.	σ			mg.	σ				σ		
Exper.	22	40.3	1.17	0.27	0.78	7.30	0.47	0.95	4.18[1]	0.183	0.004	0.14	3.93[1]
Impl. Cont.	19	39.6	2.27		0.23	7.93	0.46		5.41[1]	0.207	0.013		5.19[1]
Cont.	20	39.0	1.17			4.79	0.36			0.124	0.009		

$$\text{C. R.} = \text{Critical Ratio} = \frac{\text{obtained difference}}{(\sigma_{m1})^2 + (\sigma_{m2})^2}.$$

[1] Critical ratio is significant (greater than 3.0).

The complete weight data on the prostates of the 3 groups are shown in table 1. In our previous studies we found that there was a good correlation ($r = 0.794$) between prostate weight and body weight in immature animals of this age. Accordingly prostate:body weight ratios were also calculated for each group and included in the table.

The mean prostate weights for the two groups with implanted adrenal tissue (experimental, 7.30 mg. and implant controls, 7.93 mg.) were both significantly greater than the mean weight for the adrenalectomized castrate controls (4.79). The prostate:body weight ratios of these two groups were also significantly greater than the control ratio (table 1). These data indicate that the implanted adrenal tissues produced a substance which had a stimulatory effect on the ventral prostate. This androgenic substance, apparently passes through the liver without being inactivated since there was no significant difference in the mean prostate weights between the group in which the adrenal secretions passed directly through the liver (experimental) and the group in which the adrenal secretions did not pass directly through the liver (implant control).

Ten prostates from experimental animals and 9 from implant controls were selected at random for histological examination. According to a system previously de-

scribed (2) the conditions of the prostates were rated from 0 (complete atrophy) to +3 (normal functional state). Considerable variation was found in the prostates of both groups. In general, the smaller prostates exhibited a lesser degree of stimulation than the larger ones. One prostate from each group was completely normal; 1 experimental and 2 implant control prostates were atrophic; the rest showed various degrees of epithelial stimulation. The average rating for the experimental prostates was 1.90, while that for the implant controls was 1.61. The prostates from 27 adrenalectomized castrates, 26 days old, had an average rating of 0.11 (data from previous studies, 2).

DISCUSSION

The gross weight and histological findings clearly demonstrate that the adrenal androgen of the rat is not inactivated by the liver. In this respect if differs from the testicular androgen which, in the rat, is inactivated by the liver.

Comparing the results obtained in this study with those from our previous report (2), it is obvious that the adrenal tissue which is left in situ is more effective in maintaining the weight of the ventral prostate than the regenerated cortical tissue of the implant regardless of the situation of the implant. In the earlier report the prostates of the castrate animals with adrenals in situ averaged 125% larger than the prostates of their adrenalectomized castrate controls. In the present experiment the average prostate weights for the castrates with adrenal implants were only 52.4% (experimental) and 65.6% (implant controls) larger than their adrenalectomized castrate controls. This difference is probably due to the smaller quantity of androgen producing cortical tissue present in the animals with only one adrenal implant than in those with both adrenals left intact.

The fact that the regenerated cortex does produce sufficient androgen to cause a significant increase in the weight of the prostate clearly proves, should any doubt have arisen, that this andromimetic capacity of the adrenal is a function of the cortex and not of the medulla.

SUMMARY

The data presented indicate that the androgenic substance produced by the adrenal of the castrate immature rat is not identical with the androgen produced by the rat testes since the latter is inactivated by the liver and the adrenal androgen is not. The data furnishes no clue as to the chemical identity of this particular adrenal androgen.

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INFLUENCE OF TESTOSTERONE PROPIONATE ON SOMATIC GROWTH IN THE WHITE RAT[1]

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THIS REPORT on the growth of white rats treated over a long period with testosterone propionate is offered because the material already published (1–9) on the subject is somewhat contradictory and because considering the inhibition of such growth by estrogen, any influence by the male sex hormone in this direction is of definite interest.

MATERIAL AND METHODS

Rats of our own inbred colony, originating from Wistar rats, were treated with testosterone propionate from the first day of life as described in previous papers (14–16). One mg. was injected subcutaneously 3 times per week. Litter-mate controls were used in every experiment. The first group comprised 14 males and 46 females treated with testosterone propionate and 13 male and 18 female controls who received nothing. The second group comprised 14 males and 13 females treated while the controls, 11 males and 8 females, received sesame oil in amounts equal to the injections received by the testosterone treated animals. A third group consisted of animals treated with 5 mg. per day over a period of 30 to 60 days. Litter-mate controls received sesame oil in equivalent doses. This group consisted of 24 males and 21 female experimental animals and 19 male and 20 female controls.

All animals were kept under equal conditions of temperature and light. No constant temperature cabinet was available, but the temperature of the animal house, frequently checked. was found reasonably constant. In any case, treated and control animals were always subjected to the same environmental conditions. Food consisted of dog Purina, lettuce twice weekly, and a constantly available supply of tap water. Since no attempt was made to determine the exact amount of food consumed, no paired feeding experiments were performed. All animals were weighed weekly. Weights were charted separately for males and females, treated and controls, in every group, and then average weight charts were drawn. The number of animals from which the average weekly weight was derived was not the same every week because some litters were killed at different stages of the experiment for various studies, and because some animals, treated as well as controls, died during observation periods.

RESULTS

The weight increase of the male rats treated with 1 mg. of testosterone propionate 3 times weekly from the day of birth is shown in figure 1 as compared with their control litter mates. There is no difference between treated and controls. In figure 2 the weights of the females treated in the same way is charted as compared with their litter-mate controls. For the first six or seven weeks there is no significant difference,

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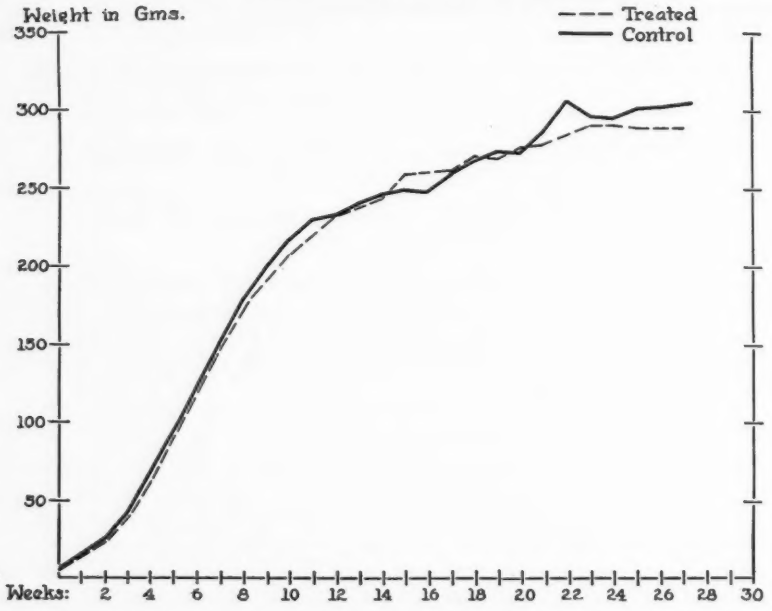

Fig. 1. AVERAGE WEEKLY WEIGHT CHART. Male rats treated with 1 mg. 3 times weekly of testosterone propionate.

then the weight curve of the treated animals increases more rapidly than that of the controls. From about the 15th or 16th week, both curves are plateaued, with the curve of the treated animals maintaining its higher level. This was true whether or not the controls had been given sesame oil.

In the last group, very large doses of testosterone propionate, 5 mg. per day, were given in order to learn whether it was possible to force an increase of growth (weight) in the first weeks of life. However, even with this high dosage, the weight curves of

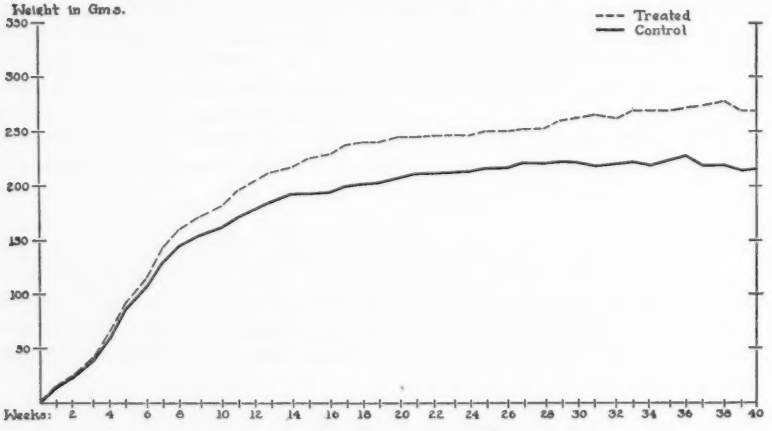

Fig. 2. AVERAGE WEEKLY WEIGHT CHART. Female rats treated with 1 mg. 3 times weekly of testosterone propionate.

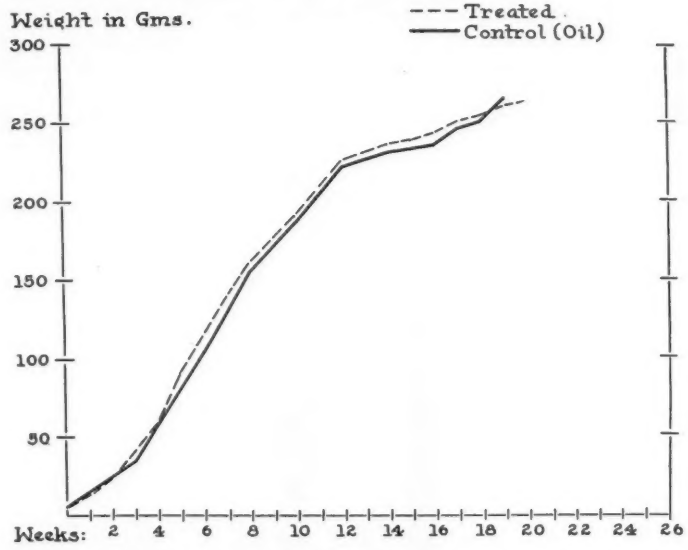

Fig. 3. AVERAGE WEEKLY WEIGHT CHART. Male rats treated with 1 mg. 3 times weekly of testosterone propionate (oil injected controls).

treated and control animals both male and female were identical in the early period (fig. 6). In fact, after 6 to 7 weeks, there was some indication of growth inhibition in the females.

At autopsy, organ weights were taken of the pituitary, adrenals, thyroids, gonads, thymus, kidney, spleen, prostate, and seminal vesicles. The weights of the

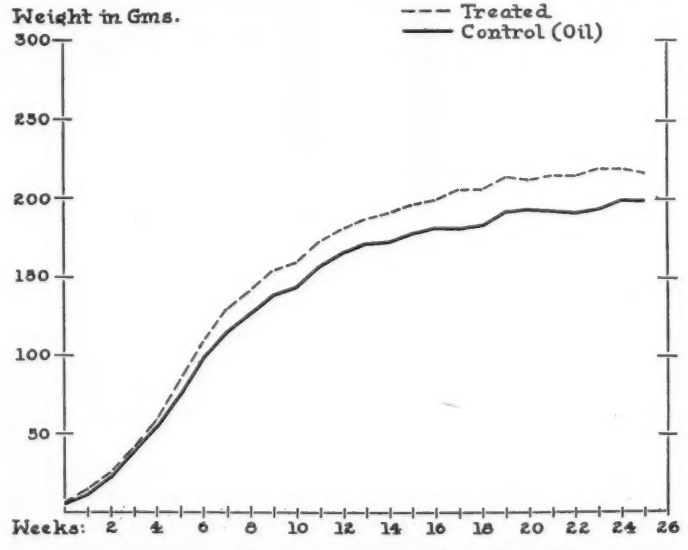

Fig. 4. AVERAGE WEEKLY WEIGHT CHART. Female rats treated with 1 mg. 3 times weekly of testosterone propionate (oil injected controls).

pituitary and kidneys only are recorded in this paper as pertinent to the problem under discussion. Because animals were killed at various stages of the experiment, and therefore at various ages, we do not record and compare the total weights but only the organ weights per unit body weight.

The pituitary weight of the female testosterone proprionate treated rat is smaller

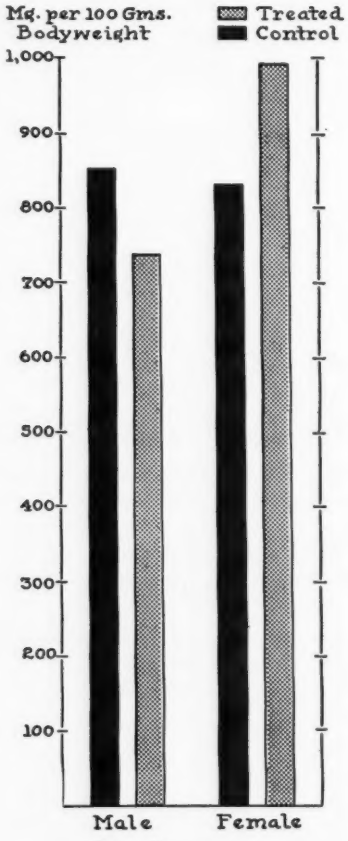

Fig. 5. KIDNEY WEIGHT.

than that of its litter-mate controls. In the male group, the pituitary weight did not change except in one litter where there was a very slight decrease (fig. 7).

The kidney weight increased in the treated females whereas it was somewhat decreased in the males (fig. 5). We have excluded from this chart all females in which the kidney weight increase was due to hydro-nephrosis or pyelonephrosis from urolithiasis which occurs in a number of females treated with testosterone propionate as described in a previous paper (15).

DISCUSSION

Weight increase as shown in figure 2 might be explained by the fact that the treated animals received sesame oil whereas their controls went without treatment. This explanation would not be supported since the female treated group only showed

weight increase. The male group treated in exactly the same way revealed no differences between treated and controls. However, the second group, represented in figures 3 and 4, were treated to eliminate this possible error. In this group, the controls received the same amount of sesame oil as did the treated animals. Again the male testosterone treated rats showed no difference in weight increase over their oil treated controls, whereas the female testosterone treated rats were heavier than their oil treated controls.

In evaluating the data, it had further to be borne in mind that a number of testosterone treated female rats carry large ovarian tumors, as described in a previous

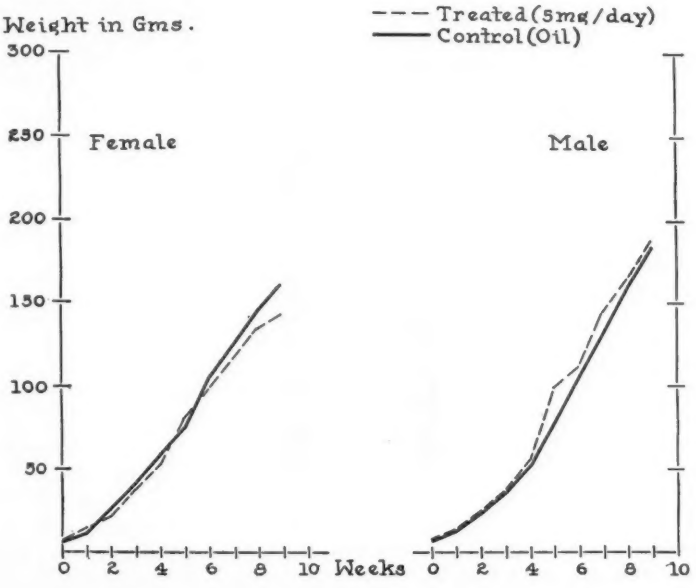

Fig. 6. AVERAGE WEEKLY WEIGHT CHART, TESTOSTERONE PROPIONATE TREATED RATS (5 MG. PER DAY, OIL INJECTED CONTROLS).

paper (14). The greater weight of the treated females might then be due to the additional weight of these ovarian tumors. This possibility was eliminated in two ways. In individual litters the weight of the ovarian tumors was deducted from the last body weight and this corrected weight compared with that of the litter mates. Averages could be taken only within each litter, not of several or all litters because autopsy was performed at different ages and ovarian weight could obviously only be deducted from the last body weight. Secondly, all treated females in which tumors were found were excluded and averages taken only from those which at later autopsy showed no tumors. Both types of calculation confirmed the findings of the first chart; namely, that the female testosterone treated rats are heavier than their litter-mate controls.

Our results are at variance with those published by Rubinstein, et al. (1) and by Kochakian (6). They are in accord with the data furnished by McEuen, Selye and Collip (4), Korenchevsky (7–9), Kochakian and Murlin (5), Kenyon, et al. (2, 3), Coffman and Koch (17), Greenhill and Freed (18). It should, however, be remembered

that dosage and time in the various experiments published by these authors differ considerably. The total dosage used by Rubinstein, et al. in rats comes closest to ours. However, they began treatment on the 26th day and injections were given daily so that their total dosage of about 50 mg. was reached in a considerably shorter time than in our experiments. Rubinstein found a significant depression of both length and weight in his experiments. Kochakian (6) found testosterone to depress the weight curve of male mice. His treatment was started at a later age than in our experiments, though still before maturity; and his dosage was considerably higher being 1.2 and 2.4 mg. per week (0.2 and 0.4 mg. per day) for a mouse as compared with 3 mg. per week for a rat in our experiment. On the other hand, Coffman and Koch (17)

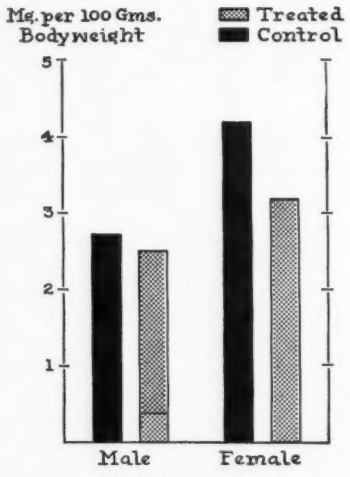

Fig. 7. Pituitary weight.

obtained weight increase in short-termed experiments on adult rats giving 0.9 mg. per day. Kochakian and Murlin (5) working on dogs found testosterone to produce small, but significant, weight increase. In the experiments of McEuen, et al. (4), the weight curves of rats treated with 0.2 to 2 mg. testosterone per day over several weeks were suggestive of stimulation in the females, and not in the males. Kenyon, et al. (2, 3) and Greenhill and Freed (18) described weight increase in human beings treated with therapeutic doses of testosterone propionate.

This weight increase is in all probability partly due to sodium and water retention (19), but in part it is undoubtedly due to changes in protein metabolism as can be concluded from the findings of nitrogen retention (2, 3, 5). The influence of male sex hormone on creatine metabolism (17, 21, 22, 23, 28), may be responsible for anabolic processes and, therefore, for weight increase; in Coffman and Koch's experiments the weight increase paralleled the decrease in creatinuria.

It is highly probable that the influence of testosterone propionate on the weight increase is exerted through the pituitary. The metabolic changes as induced by male sex hormone (2, 3, 5) resemble those produced by administering anterior pituitary extracts (24–29). Both anterior pituitary extracts and testosterone propionate produce a positive nitrogen balance; namely, storage of protein. The decrease of creatinuria as induced by male sex hormone has its parallel in the decrease of creatinuria due to anterior pituitary extracts (30). A definite proof that testosterone propionate exerts

its metabolic and growth effects by way of the pituitary can be adduced by studying the effect of testosterone propionate in hypophysectomized animals.

Testosterone propionate markedly decreases the pituitary weight of female rats when given in large doses over a long time (14, 31, 32).

In males, however, as has been stated in this paper, testosterone does not influence pituitary weight. Histological study of our own material is not yet completed. Of the manifold changes in the histological structure of the pituitary of animals treated with androgens as described by various authors, the findings of Wolfe and Hamilton (32), of an increased level of eosinophilic cells are of particular interest. There can be little doubt that the eosinophils of the pituitary are related to the growth promoting function. The female pituitary under the influence of male sex hormone approaches the male pattern. Concomitantly the growth curve of the treated female rat approaches that of the male. It never exceeds the latter: giant rats are not produced by testosterone treatment.

Another possibility to be considered is an indirect action of the testosterone propionate. Estrone is known to inhibit pituitary function, both gonadotropic and growth. Testosterone propionate depresses ovarian function and the lack of estrone secretion could secondarily release the pituitary and thus produce an increased output of growth hormone.

At autopsy we found a very definite splanchnomegaly in the testosterone treated female rats. We have determined the kidney weight as an indicator of splanchnic enlargement. Owing to the fact that animals varied considerably in age and weight at the time of autopsy, a comparison of absolute organ weights would be of no significance. Accordingly, we calculated the organ weight per unit of body weight. The kidneys of the treated females were heavier than those of the controls (fig. 5). Splanchnomegaly is a well-known finding in acromegaly and has also been found in animals treated with anterior pituitary growth hormone (33). In these acromegalic animals, the weight of the viscera was increased more than the body weight. An increase of the weight of the kidney in testosterone treated mice has recently been reported by Selye (34).

Again it should be noted that the splanchnomegaly as indicated by relative weight increase of the kidney was present only in the females and not in the males, thus offering another indication that only in the females does growth stimulation through testosterone propionate take place (fig. 5).

Comparison of the growth curves of the treated and control females reveals that the increased growth can be detected at about 6 weeks. Within the first 4 or 5 weeks, there is no difference in the rate of weight increase of treated and control animals. This can be due either to intrinsic factors, such as reactivity of the pituitary to the testosterone or the organism to the output of pituitary growth hormone or it can be due to insufficient dosage. Increasing the dosage to 30 mg. per week (instead of 3 mg. per week) did not in any way change the weight curve within the first 4 weeks (Fig. 6). It can hence be concluded that weight increase is not only a question of dosage. The period of treatment, or possibly the reactivity of the organism, plays the decisive role.

Continuing the heavy treatment, 5 mg. per day 6 times per week over 8 weeks, there is even an indication of impaired growth in the females (fig. 6). Our figures, however, are not conclusive because the number of animals observed over this period was too small.

We wish to point out that some findings already quoted above are apparently at variance with both the facts and the explanation of our experiments. Whereas, we have found weight increase only in the females treated with testosterone propionate,

testosterone does seem to lead to weight increase also in male rats (17), and in men (2, 3). As a matter of fact, most of the metabolic effects of testosterone propionate upon which we have based the discussion of the weight increase have been studied in male individuals. As things stand at the moment, we are at a loss to explain the difference of reaction of males in our experiment with those reported by other authors. It should, however, be pointed out that dosage and timing in our experiments were different from those in which positive results were obtained.

Finally, it should be noted that both female and male sex hormone inhibit the output of pituitary gonadotropic hormone, at least as far as FSH is concerned. The LH seems to be stimulated in the female under certain circumstances (14). But apart from that the action of testosterone propionate is apparently the opposite of that of estrogen. Estrogen leads to an increase of pituitary weight and finally to formation of adenomas, testosterone decreases pituitary weight. Estrogen inhibits the output of growth hormone,[2] testosterone stimulates it.

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[2] We have spoken in this paper of growth hormone as the hormone responsible for weight increase and the underlying metabolic processes. There are some doubts as to the identity of this 'protein metabolism hormone' with a true growth hormone (35).

METABOLISM OF HUMAN SPERM[1]

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THE PURPOSE of this report is to present the results of an investigation of the nature and extent of metabolism of human sperm. The seminal specimens were obtained from the Vanderbilt Clinic and the Sloane Hospital, and from cases under the care of private practitioners. The series therefore included a large proportion of samples from men who might be suspected of impaired fertility or of other abnormalities of sperm. On the basis of clinical findings and case histories the men providing the specimens were classified into three categories in respect to fertility and normality. It is obviously difficult to be accurate in determining such categories. In cases of fertile marriage, with normal children, the man was considered as being normally fertile (*series A*). When a marriage was sterile but pelvic examination of the wife showed sufficient abnormality to account for the sterility the male, if apparently normal, was classified as probably fertile (*series B*). In those cases in which the marriage was not fertile, or successive pregnancies were interrupted by miscarriage or produced fetal anomalies, and in which the wife showed insufficient pathology to account for the history, the male was classified as being suspected of impaired or disordered fertility or abnormality (*series C*). It is evident that this third group must be larger than it would be if we were able to determine accurately the true fertility. Fertility is a relative term at best, and its definition in human biology is difficult. It can not be safely assumed that a sperm which appears under the microscope as motile and morphologically normal must be normal. Defects in sperm which might cause inability to reach the Fallopian tube, or to fertilize an ovum, or to produce normal development of the ovum, may not be subject to assay by present methods. We have therefore chosen to exclude from the normal group all those cases in which there may be any clinical reason to suspect abnormality. An attempt was made to determine whether this third category, indicated as "suspected of impaired fertility," showed any detectable metabolic deviation from the normal.

Morphological examinations of the sperm were also made, but the results were not used in determining the categories. These data will be discussed in a later paper.

We are not familiar with the existence of any data dealing with the metabolism of sperm from abnormal specimens of semen and there is relatively little information concerning the metabolism of human sperm from fertile semen. The earlier work of McCarthy et al. (1), demonstrated a disappearance of sugar and an increase in lactic acid on incubation of semen containing motile sperm. The more recent experiments (2, 3) have been performed with the technic of Warburg. The former investigator

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found that the fresh sperm from young, healthy subjects glycolyze aerobically, producing sufficient lactic acid to yield about 0.080 mm.^{3} CO_2 per 10^6 cells per hour, when measured in Ringer-bicarbonate solution equilibrated with 5% CO_2 in O_2. He found so little oxygen uptake in Ringer-phosphate solution of pH 7.4, equilibrated with air or oxygen that he was not convinced that there was any utilization of molecular oxygen. Shettles reported that sperm respire in semen. Using the direct method of Warburg he found an average uptake of 10.4 mm.^{3} per hour by 75×10^6 sperm over a 3-hour period in a ½-hour-old specimen, and approximately twice as much by 150×10^6 sperm in another sample. The value dropped to 7.2 mm.^{3} for 75×10^6 sperm in a 6-hour-old semen. No measurements of glycolysis were reported. The data of MacLeod and of Shettles are not in agreement regarding respiration, but the menstrua were different; moreover in the latter's experiments the pH of the fluid was higher than physiological owing to the removal of CO_2 by alkali. Shettles reports respiratory quotients of 0.93 for ½-hour-old specimens and 0.72 for a 12-hour-old sample, using Dickens and Šimer's first method in which the CO_2 tension also approaches zero.

A number of investigators (4–14) have measured the metabolism of sperm other than human. Redenz (7) reported that bull's spermatozoa take up oxygen at the rate of 6 mm.^{3} per mg. (the weight of 37×10^6 dried sperm) per hour in inactivated serum dialyzed against Ringer solution containing 25 millimols bicarbonate per liter, and 9.0 mm.^{3} in the same serum to which glucose had been added. There was considerable variation in the intensity of respiration among different specimens. Glycolysis yielded 14.7 mm.^{3} CO_2 (average) per hour per mg. (dry basis). These values were obtained during a 30-minute run. Iwanow (8) reported R.Q.'s of 0.78 and 0.79 for ram sperm in Ringer solution without glucose over a period of 70 minutes, and R.Q.'s of 0.90 and 0.96 when glucose was added. Glycolysis was not measured. Winberg (14) reported an R.Q. of 0.92 for avian sperm.

In most of the specimens studied, we have determined bicarbonate and reducing substances. Goldblatt (15), Huggins and Johnson (16), and others have published the results of quantitative determinations of these constituents of normal semen.

Volume of semen, sperm count, percentage of motile sperm, and relative motility were observed and recorded. Bacterial counts (blood agar, 37°C.) were made on many of the specimens in order to obtain some idea of the extent of contamination, and of the possible contribution of microorganisms to the measured metabolism. The counts ranged from 500 to 500,000 per cc. but showed no suggestion of correlation with nor effect upon the experimental results.

METHODS

Sterile glass containers were supplied and instructions given to collect the specimen following interrupted coitus.

Metabolism. The second method of Dickens and Šimer (17), with Brodie fluid in the manometer, was used for measuring glycolysis and respiration simultaneously in Ringer solution. Sterile pipettes were used for measuring and transferring. Seminal fluid was centrifuged at 615 G and the cells resuspended in sterile Ringer-bicarbonate solution which had been equilibrated with 1.2% CO_2 in O_2. When in the Ringer solution at room temperature the sperm showed normal or occasionally enhanced motility. The cell suspension was placed in the manometer vessels in the water bath at 37.5°C., a mixture of 1.2% CO_2 in O_2 was passed through for 10 minutes, and then 5 minutes were allowed for final equilibrium. During this time and for the remainder of the experiment the vessels were shaken. The calculated pH was about 7.6 at the start. Several tests were made with the cells suspended in the subject's own

seminal plasma, in which the bicarbonate had been partially neutralized by 0.1 N HCl in order to bring the manometric excursions within readable limits. Here also 1.2% CO_2 in O_2 was employed. Retention of lactic acid (by protein, etc.) produced by glycolysis was corrected for by the procedure of Dickens and Šimer for retention by

TABLE 1. METABOLISM OF SPERM SUSPENDED IN RINGER-BICARBONATE SOLUTION

Subject No.	Classification	Vol. of specimen	Elapsed time	Hanging drop		No. of sperm per cc.		Glycolysis per hr. per 10^8 sperm in mm.^{3} CO_2
				Speed	% Motile	Original $\times 10^{-6}$	In expt. $\times 10^{-6}$	
		cc.	*hours*					
3	A	2.2	4	4+		390	250	10.4
4	A	3.5	4	3+		97	91	16.8
8	A	3.8	3	2 to 3+	75	89	57	21.6
9	A	6.0	4	1 to 2+	30	84	92	18.0
11	A	1.2	3	3+	75	—	26	33.2
20	A	7.5	3.5	1 to 2+	25	45	57	7.7
25	A	—	3	3+	75	32	10	13.5
1	B	3.2	4	3+		119	88	16.6
5	B	2.3	3	4+	100	243	132	19.4
6	B	4.0	3	4+		297	198	9.8
10	B	4.0	3	2 to 3+	75	68	84	13.8
17	B	2.8	—	—		136	100	17.7
21	B	3.0	3.5	1+	75	42	29	23.5
21*	B	4.8	4	2+	75	40	29	19.8
		Same specimen as line above‡						20.2
21†	B	3.4	4	1 to 2+	50	30	20	18.8
		Same specimen as line above§						18.2
22	B	2.3	20	1+		124	48	7.3
24	B	1.5	5		5	88	27	14.6
26	B	5.7	4	3+	90	102	88	24.8
27	B	4.0	4	2+	50	29	22	16.8
28	B	3.2	4	3 to 4+	90	94	58	15.8
30	B	5.3	—	3+	30	22	23	22.8
2	C	3.0	2	3+		—	55	33.0
7	C	—	3	4+		22	29	14.5
12	C	3.0	3.5	2+	75	—	55	7.9
13	C	4.3	3.5	2+	75	—	165	5.8
14	C	3.0	9	4+	90	—	90	18.1
15	C	2.0	3	4+	50	110	59	22.4
16	C	2.3	3.5	1+	5	153	76	8.7
18	C	3.5	—	2+	50	77	38	19.9
19	C	1.3	5	4+	90	82	22	30.9
23	C	5.5	4	2+	75	92	83	17.5
29	C	6.0	6	4+	90	89	92	14.3

A = Definitely fertile.
B = Probably fertile.
C = Probably impaired fertility.
* 1.2% CO_2 in O_2 and 0.4% glucose.
† 1.2% CO_2 in O_2 and 0.2% glucose.
‡ 4.5% CO_2 in O_2 and 0.4% glucose.
§ 1.2% CO_2 in O_2 and 0.4% glucose.

serum, in which a curve relating the drop in pH with CO_2 retention by a sample of pooled seminal plasmas was employed.

Two experiments were made on cells resuspended in human follicular fluid[2] (obtained from a follicular cyst) after partial neutralization of the bicarbonate and under 1.2% CO_2 in O_2. The rate of motion of the sperm in this fluid and in the modified

[2] The original bicarbonate content of the fluid was 0.0226 mols per liter. (equilibrated with 1.2% CO_2). Chlorides equalled 653 mg. per 100 cc. (as NaCl) and reducing substances 67 mg. per 100 cc. (as glucose).

semen was not noticeably different from that in unaltered semen. The quantity of lactic acid retained was determined as before; the retention was small (about 10 mm.³).

A fourth group of experiments was carried out in the subject's unaltered semen and in an atmosphere of pure oxygen. Dickens and Šimer vessels were used and with few exceptions 1.5 cc. of the specimen employed. After final equilibration in the water bath the NaI and $KMnO_4$ were mixed in one vessel containing semen in the inner cup in order to absorb CO_2. A vessel in which these reagents were similarly mixed, and containing Ringer-bicarbonate (0.006 M per liter), served as a thermobarometer. This is important as the readings in such a thermobarometer increased at the rate of 3.5 to 4.0 mm. per hour over that of a vessel in which these reagents were

TABLE 2. METABOLISM OF SPERM IN FOLLICULAR FLUID AND MODIFIED SEMEN
(Nos. 31 and 32 were done in Follicular Fluid; all others in Modified Semen)

Subject No.	Classification	Vol.	Elapsed time	Hanging drop		No. sperm per cc. $\times 10^{-6}$		Glycolysis per hr. per 10^8 sperm. In mm.³ CO_2‡	Oxygen uptake per hr. per 10^8 sperm. In mm.§
				Speed	% Motile in semen	Original	In expt.		
		cc.	*hours*						
6	B	3.5	2	4+	95		145	25.6	2.8
21	B	6.5	1	3 to 4+	80		82	21.3	*
34†	B	3.1	1.5	4+	95		126	28.9	4.4
35†	B	3.5	5	4+	90				
19	C	2.9	4.5	3+	90	84	77	12.6	1
31	C	3.9	3.5	2 to 3+	50	108	91	9.8	
32	C	2.5	3	4+	90		68	13.9	
33	C	3.3	4	3+	50		118	14.4	1

For meaning of classification symbols see table 1.

* Total volumes measured were 11.4, 9.8 and 5.4 mm.³. Because of the small total quantities the hourly rate is not recorded.

† Experiment made on pooled 34 and 35.

‡ Corrected for retention.

§ Uncorrected for possible O_2 uptake by the modified plasma alone.

kept separate. Later it was also found that 1.5 cc. of seminal plasma free of sperm takes up some oxygen. Five different specimens of plasma were examined and the average O_2 uptake was 13.0 mm.³ for a 3-hour run, about ¾ of this being consumed in the first 2 hours. Hence 13 mm.³ were subtracted from the readings in the sperm oxygen uptake experiments in which the uptake by plasma alone had not been measured. This resulted in the finding that several sperm specimens did not respire. In 3 experiments oxygen uptake by plasma alone was determined at the same time as it was measured for the sperm derived from the same specimen of semen and was used for calculating the net uptake by the cells. When glycolysis was also to be measured a third vessel containing semen and unmixed NaI and $KMnO_4$ was set up; a fourth one with unmixed NaI and $KMnO_4$ and Ringer-bicarbonate (6 millimols per liter) served as its thermobarometer. The readings on the manometer attached to the third vessel were resultants of the oxygen consumption, respiratory CO_2, and CO_2 liberated by glycolysis lactic acid acting on bicarbonate. We assumed an R.Q. of 0.92 in calculating glycolysis. Manometric changes which occurred during the first 15 minutes following completion of a 15-minute equilibration period were disregarded. Owing to the retention of some respiratory CO_2 and some lactic acid, neither of which was corrected for, the recorded glycolysis value is too low.

Only in this fourth group of experiments was it possible to examine the sperm for motility at the conclusion of the test and in almost every case it was considerably reduced. The varying rate at which motility diminished probably contributed to the

large differences in oxygen uptake observed in different specimens.[3] In the other experiments the method required the addition of HCl to the sperm suspension at the conclusion of the run. The usual duration of the tests was 3 hours; occasionally it was 2 or 4 hours. Sperm retained their motility fairly well when suspensions in Ringer-bicarbonate solution equilibrated with 1.2% CO_2 were shaken in the water bath for 3 hours to simulate the conditions of the experiments carried out in this medium.

Reducing substances in seminal plasma. Plasma, 0.20 cc. was added to 1.8 cc. Folin-Wu reagent of double strength. Water clear filtrates were obtained. Reduction

TABLE 3. METABOLISM OF SPERM IN UNALTERED SEMEN

Subject No.	Classification	Vol.	Elapsed time	Hanging drop		No. sperm per cc. $\times 10^{-6}$	Glycolysis per hr. per 10^9 sperm. In mm.^{3} CO_2*	Oxygen uptake per hr. per 10^8 sperm. In mm.^{3}
				Speed	% motile			
		cc.	*hours*					
25	A	4.0	12	3+	75	183		1†
40	A	3.5	2.5	2 to 3+	70	197	6	3‡
10	B	5.0	3.5	3+	80	159		1‡
22	B	2.0	2	3 to 4+	95	51		13‡
26	B	6.0	3	3+	65	50	21	9‡
37	B	4.5	1.5	3 to 4+	65	280	4	
37	B	4.0	2.5	4+	75	194	4	3‡
38	B	3.0	2.5	4+	80	176	7	4‡
38	B	2.0	3	3 to 4+	90	232		3‡
41	B	3.5	3	3 to 4+	80	76	7	9‡
45	B	6.0	4	3+	90	37		n.r.§
33	C	2.9	4.5	3+	75	81	8	n.r.
36	C	1.2	1	4+	90	235	8	
39	C	1.0	2.5	4+	85	110	10	
42	C	2.2	5.5	2+	20	80	7	n.r.
43	C	4.5	7.5	2+	50	47		n.r.
44	C	3.5	7	3+	25	140		n.r.
46	C	4.1	6	4+	90	94		5†
47	C	4.0	5	4+	80	120		2†

For meaning of classification symbols see table 1.
* Uncorrected for retention.
† Corrected for blank made on seminal plasma of same specimen.
‡ Corrected for average value of blank made on several seminal plasmas.
§ n.r. = No respiration after correction for average oxygen uptake by sample of pooled plasmas alone.

of copper was measured colorimetrically after adding phospho-molybdate. The results were calculated in terms of glucose.

Bicarbonate. This was determined after equilibrating with 1.2% CO_2 in O_2, in Dickens and Šimer vessels using a 1:7 dilution of the plasma.

Motility. Vaseline sealed hanging drops were examined under the high, dry objective while focusing on several planes which often displayed different appearances. The more sluggishly moving and non-motile sperm were more frequent in the lower levels. Examination was made soon after samples reached the laboratory; most of the specimens were delivered between one and two hours after collection.

RESULTS

Glycolysis. Our results for those experiments which were made in Ringer-bicarbonate solution are qualitatively the same as MacLeod's, the metabolism being pre-

[3] Sperm retained their motility much better at room temperature and in air than they did at 37.5°C. and under pure O_2.

dominantly glycolytic in the presence of oxygen. No difference between the metabolism of sperm from 'definitely fertile' or 'probably fertile' men and those men of 'impaired fertility' appears to exist. High and low glycolysis figures were obtained in both groups. The range was from 5.8 to 33.2 mm.^{3} CO_2 per hour, per 10^8 sperm. With few exceptions our figures are considerably larger than MacLeod's average. The difference is probably not due to the fact that he used 4.5% CO_2 while we employed 1.2% nor to the increase in glucose concentration from 0.2 to 0.4%. We measured the metabolism of one individual's sperm in the presence of 0.2% glucose as well as under 4.5% CO_2, and obtained results very similar to those secured with the lesser concentration of CO_2 and larger quantity of sugar. The low values for glycolysis (5.8 to 8.7) were obtained for 5 specimens in which motilities were graded as 2+ or less, and of which 75% or less were motile. However, values from 16.8 to 22.4 were observed in 8 other specimens graded 1+ and 2+. It is known that sperm, in different semens, which are at the start equally motile, cease moving after varying intervals of time. This might partly explain the difference just noted. Comstock (5) found that the rate of glycolysis by ram sperm "is closely related to their ability to remain motile when stored." Fifteen specimens whose motilities were graded as 3+ or 4+ had, with two exceptions, moderate to high glycolytic values (14.3 to 33.2). The lowest two rates (10.4 and 9.8) were obtained for two specimens in which the densities of population were high, 250×10^6 and 198×10^6 per cc. Gray (11) has observed a greater rate of oxygen uptake by dilute than by concentrated suspensions of sperm of *Echinus esculentus* and *E. miliaris*. He noted also that the failure of these sperm to move in testicular fluid could be explained by the fact that they were too crowded; simple dilution with seminal plasma was followed by intense activity. The total volumes of carbon dioxide due to glycolysis (ranging from 17.0 to 115.5 mm.^{3}) were, with few exceptions, sufficiently large to warrant confidence in the results. We believe that the total quantities over 15 mm.^{3} CO_2 are trustworthy.

Glycolysis in semen in which part of the bicarbonate was neutralized proceeded at about the same rate as in Ringer-bicarbonate solution. The figures were 14.4, 28.9, 12.6, and 25.6. The two low values were for specimens in which the motility, though good, was less than in the other samples. The specimen (*case 21*) with the value of 21.3 was one of four from the same individual. The other three, all of which were definitely less motile, were tested in Ringer-bicarbonate solution and produced 23.5, 19.8 and 18.8 mm.^{3} per hour, per 10^8 sperm. Another subject's sperm (*case 19*) yielded 30.9 mm.^{3} in Ringer-bicarbonate solution and only 12.6 mm.^{3} in modified semen although the speed of motion in the second case was only slightly less than in the first.[4] The greatly reduced sperm population in all three of the first subject's specimens which were tested in Ringer-bicarbonate solution and in the second subject's specimen similarly tested, as contrasted with the normal populations in the modified semen experiments may be the explanation for the relatively higher glycolysis values in the former medium. However, a third man's sperm (*case 6*) produced 25.6 mm.^{3} glycolysis CO_2 per hour per 10^8 sperm in modified semen and only 9.8 mm.^{3} in Ringer solution although neither population densities nor speeds of motion were very different.

Values of 9.8 and 13.9 were obtained for two specimens examined in human follicular fluid, the bicarbonate content of which had first been reduced from 22.6 to 8 millimols/liter by the addition of 0.1 N HCl. The sperm which glycolyzed at a slower rate moved more slowly; also the percentage of motile sperm was less.

Uncorrected glycolysis values in unaltered semen ranged from 4 to 10 mm.^{3} per

[4] First test was made Feb. 5; second April 22. During November and December this subject had received 1500 I.U. gonadotrophic hormone once a week for 3 weeks. From March 10 to April 22 he received 1500 I.U. gonadotrophic hormone 3 times a week.

hour per 10^8 sperm with the exception of one specimen (*case 26*) which yielded 21 mm.^{3} A specimen from the same person yielded 24.8 mm.^{3} per hour for the same number of sperm in Ringer-bicarbonate solution. The rate was not always greater for those sperm moving faster.

Respiration. In experiments carried out in Ringer-bicarbonate, follicular fluid and modified semen the determination of respiratory CO_2 and O_2 uptake by the Dickens and Šimer method is subject to considerable error when the respiration is small. This is substantiated experimentally by the fact that several negative values were obtained for respiratory CO_2 the largest of which were -6.2, -8.5, -10.7, and -7.4 mm.^{3} (total volumes). We have therefore, calculated the rate of O_2 uptake for only the largest total volumes measured in the experiments performed in Ringer solution (*cases 5*, 6 and 9) which were 19.2, 31.1, 17.9 mm.^{3} O_2, or 3.2, 3.5, and 4.3 mm.^{3} per hour per 10^8 sperm. These 3 specimens were from men who were either definitely fertile or probably fertile. The remaining specimens in these two groups of subjects did not take up oxygen.

Two of the five values for total respiratory oxygen uptake in the modified semen experiments were 26.7 and 23.8 mm.^{3} and amounts consumed per hour per 10^8 sperm were 4.4 and 2.8 mm.^{3} The last figure was that of a subject (*case 6*) who was one of the three whose sperm, when examined in Ringer solution, yielded a value for oxygen consumed considered reliable (3.5 mm.^{3} per hour per 10^8 sperm). The total quantity of O_2 consumed in each of the remaining experiments was considered too small to inspire confidence in the result of an hourly rate calculation. The same applies to the single determination in which respiration was measured in follicular fluid.

In those experiments which were done in unaltered semen the conditions appear to be such as to yield reliable results, except for the fact that the pH is higher than physiological. The hourly consumption per 10^8 sperm (corrected for the average quantity of O_2 taken up by seminal plasma alone) lay between 3 and 9 mm.^{3} per hour per 10^8 sperm with 2 values of 1 and 1 of 13. Determinations on two different specimens from the same individual (*case 38*) yielded 4 and 3 mm.^{3} A specimen from another subject (*case 26*) yielded 9 mm.^{3} in unaltered semen and 3.5 mm.^{3} in Ringer solution. The latter value (not shown in the table) was, however, calculated from a total O_2 consumption of only 13.7 mm.^{3}, a figure which, as explained above, may have a large error. The larger value was obtained at the same time as a high glycolysis figure. Our figures for O_2 consumption are, in general, lower than those of Shettles. In general the low O_2 uptake values were obtained in these experiments in which the sperm populations were high and the high values where the populations were low. The sperm from subject (*case 22*) had the highest respiration of any specimen examined in seminal plasma but consumed so little O_2 in Ringer-bicarbonate that the rate was not calculated. This may have been due in part to the age of the specimen examined in the artificial medium (20 hr.).

An attempt to learn whether oxygen would be consumed by sperm suspended in Ringer solution containing 20 millimols bicarbonate per liter and adjusted to an initial pH of about 8.0 and equilibrated with pure O_2 yielded total O_2 consumption values so low that they were considered unreliable. Sperm motility was immediately and profoundly reduced in this solution.

Number of sperm. There was no significant correlation between the sperm count and the clinical classification. No sperm were seen in one uncentrifuged sample from each of two definitely fertile men, illustrating the variations in sperm population at different times in an individual's semen and the need for examination of more than one sample when very few sperm are found. In the case of one of these two men other specimens contained 175×10^6, 81×10^6 and 183×10^6 sperm per cc.

Bicarbonate and reducing substances. The bicarbonate determinations gave mean values, with standard deviations, of 20.6 ± 8.4 millimols per liter (range, 4.0 to 35.7) for 20 fertile and probably fertile cases, and 19.3 ± 7.6 (range, 4.9 to 36.3) for 16 cases suspected of impaired fertility. The reducing substances (calculated as glucose) gave averages of 399 ± 97 mg. per 100 cc. (range, 180 to 615) for 21 cases of the first two groups, and 508 ± 159 (range, 175 to 755) for 16 cases of the third. In neither item was there statistical significance in the differences between the series. There did not appear to be any correlation between the individual data and the volumes of the specimens, cell counts, motilities, metabolic rates or clinical findings.

DISCUSSION

The data indicate that sperm from all three groups of men glycolyzed aerobically whether sperm were suspended in Ringer solution, in follicular fluid or in semen. Quantitative differences existed within each group probably owing largely to the rate of motion of the cells and the percentage which were motile. Quantitative differences in glycolysis between groups are consequently impossible to detect.

It is difficult to decide from the data whether there is a difference in respiration between sperm from definitely fertile and probably fertile men and those from men in the impaired fertility group. Although all 3 apparently reliable cases of oxygen uptake in the Ringer experiments were in the first two classifications, all the remaining experiments with such sperm in this fluid failed to show evidence of oxygen consumption. Of the 5 experiments which were carried out in modified semen 2 show that oxygen was consumed. These specimens were both from men considered as probably fertile. No correction has been applied to this group of figures in the table for possible oxygen uptake by the medium itself such as was observed in unaltered seminal plasma.

Sixteen respiration experiments were made with unaltered semen. Six of these were made on specimens from men who were considered probably infertile; two of these (*case 46* and *case 47*) consumed enough oxygen, 22 mm.^{3} (total volume after correction), to make it appear that the measurements were reliable, the remaining 4 took up total quantities which were the same as the average amount taken up by plasma alone. Of the 10 specimens in the definitely fertile and probably fertile groups, only one (*case 45*) took up so little oxygen (uncorrected) as to cast doubt on its accuracy; two others (*cases 10* and *25*) consumed only 11 and 12 mm.^{3}, after correction for the uptake by plasma alone. The remaining seven consumed oxygen.

In general it would appear that sperm from men in the fertile and probably fertile groups take up more oxygen than sperm from men who were considered as possibly infertile, but more data must be obtained before this can be accepted as proven.

In a paper by Chang and Walton (18) which has come to our attention since this manuscript was written reference is made to articles by Walton and Edwards (19) and Walton (20) in which it was shown that the respiratory activity can be used as a criterion of sperm activity, which is closely related to the fertility of the male (sheep, cattle and horses).

SUMMARY

Glycolysis and respiration of human sperm were measured simultaneously. Sperm from men who were definitely fertile, probably fertile, and suspected of impaired fertility, all glycolyzed aerobically in Ringer-bicarbonate, in human follicular fluid, and in semen. Quantitative differences could not be detected among the three groups. Oxygen uptake was observed in only 3 instances out of 30 experiments made with Ringer-bicarbonate; all 3 specimens were from men in the first two groups. Of 5 tests made with modified semen 2 showed evidence of slight oxygen consumption, both

specimens being from men considered probably fertile. Lesser quantities, considered within the range of experimental error, were consumed by 3 specimens from men considered probably infertile. The sperm from 7 men out of 10 in the first two groups took up oxygen in unaltered semen, and the cells from 2 out of 6 men in the third group did so. However, additional experiments would be necessary to establish a difference in respiratory metabolism of sperm from men known to be fertile and sperm from men suspected of impaired fertility when the cells are examined in unaltered semen.

There are indications that degree of motility and density of sperm population may be in some cases major factors in determining the metabolic rate.

No significant differences in content of bicarbonate and reducing substance were observed in the seminal plasmas of the two main groups of men.

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THE INACTIVATION OF ESTRADIOL AND ESTRADIOL BENZOATE IN CASTRATE FEMALE RATS

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THE IN VIVO TECHNIC of studying the inactivation of certain estrogens and androgens has disclosed that when estrone (1) and the semisynthetic compounds, testosterone propionate (1) and methyl testosterone (2), are implanted as a pellet in the spleen, they are unable to exert their specific action on the castrate animal of the appropriate sex. The site of inactivation has been considered to be the liver. Additional evidence that the liver plays an important role in this process has been brought out by numerous observers (3, 4). Both Zondek (5) and Heller (6) found that estradiol is inactivated when incubated with liver slices or minced liver. The present experiment was designed to determine if estradiol and estradiol benzoate are inactivated by the liver in vivo.

METHODS

Compressed pellets of estradiol[1] and estradiol benzoate[1] were prepared in a specially designed mold, under a pressure of 6,500 lb. per sq. inch (7). The pellets varied in weight from 1.1 to 1.8 mg. Twenty-eight adult female rats were castrated and used only when they were found to be in a permanent anestrous state. The pellets were implanted in the subcutaneous tissues of one group, and in the spleens of another group. In some of the latter group the spleen was transplanted between the overlying muscles and the skin. In the animals receiving a pellet of estradiol, it was necessary to place a ligature around the splenic vessels two weeks after the transplantation to prevent the splenic blood from reaching the liver. Vaginal smears were examined daily.

RESULTS

A pellet of either estradiol or estradiol benzoate produced continuous estrus when implanted in the subcutaneous tissues of the castrate female rat. When implanted in the spleen, it effected an immediate short irregular estrous smear which regularly changed to a permanent anestrous smear.

When the spleen containing a pellet of estradiol benzoate was transplanted, thus causing the development of an extra-portal collateral circulation, estrus appeared on the fourth day and continued for 50 days. To obtain a continuous estrus with estradiol, it was necessary after transplanting the spleen containing the pellet to ligate the splenic vessels. The results are given in table 1, with details concerning the number of animals used in each group, the exact duration in days of each experiment, and the average amount of each substance absorbed per day.

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[1] Supplied through the courtesy of Ciba Pharmaceutical Products Inc

Histologic examination of the transplanted spleens showed no significant alteration in the structure of the spleens bearing estradiol benzoate pellets; the vessels of these spleens were not ligated. The transplanted spleens bearing the estradiol pellets, whose vessels had been ligated, showed no areas of infarction, but the pulp contained an increased number of large mononuclear cells. This change had been noted before (1).

DISCUSSION

These results show that a small pellet of either estradiol or estradiol benzoate when implanted in the subcutaneous tissues is slowly absorbed and produces a constant estrus in the castrate female rat. When the pellet is implanted in the spleen, there is an immediate short irregular period of estrus that is always followed by an uninterrupted period of anestrus. Absorption from the pellets is approximately the same from either the subcutaneous tissues or the spleen. The compounds absorbed in the spleen

TABLE 1.

Site of implantation	No. of animals	Immediate effect	Permanent effect	Av. daily abs., mg.	Subsequent procedures and effects
			ESTRADIOL		
Subcutaneous tissues	4	Estrus	Estrus—70 days	0.011	None
Spleen	5	Estrus—4 days[1]	Anestrus—66 days	0.007	None
Spleen	4	Estrus—10 days[1]	Anestrus—32 days	0.010	Spleen transplanted; incomplete estrus for 14 days. Splenic pedicle ligated: permanent estrus for 28 days.
			ESTRADIOL BENZOATE		
Subcutaneous tissues	3	Estrus	Estrus—80 days	0.007	None
Spleen	5	Estrus—18 days[1]	Anestrus—61 days	0.007	None
Spleen	7	Estrus—18 days[1]	Anestrus—13 days	0.006	Spleen transplanted: estrus appeared on 4th day. Permanent estrus without ligating pedicle for 50 days.

[1] Longest period observed in any animal in the group.

are carried in the blood stream to the liver, and pass through the liver before reaching the organs they specifically affect. This passage through the liver apparently prevents estradiol and estradiol benzoate, like estrone, from producing their specific effect on the vagina and uterus. If the blood leaving the spleen containing the pellets is diverted from the liver to the general circulation, estrus immediately becomes apparent. This collateral anastomotic circulation, developed by transplanting the spleen, causes sufficient estradiol benzoate to enter the systemic circulation to produce constant estrus. However, to obtain the effect with estradiol, it is necessary to place a ligature around the splenic vessels, so that the collaterals develop more fully and all the absorbed estradiol enters the systemic circulation. This slight difference in action between the pure compound and its benzoate may signify that when absorbed from pellets in these rats, the benzoate is more potent, or that it is somewhat less rapidly destroyed by the liver. Another possibility, but less likely, is that a greater collateral anastomotic circulation developed in the animals whose spleens contained the pellets of estradiol benzoate.

Heller (6) has recently found that before inactivating estrone, liver tissue slices and liver tissue mince appear first to convert it to estradiol, whereas there is immediate inactivation of estradiol. The present in vivo experiments showed no apparent

differences in the site or mode of inactivation of estradiol and estradiol benzoate, as compared with estrone.

The short period of estrus that occurs immediately after the pellet of either estradiol or estradiol benzoate is introduced into the spleen was also noted in the experiment with estrone (1). This period of estrus is variable, the greatest duration for any one animal in each group being 14 days for estrone, 18 days for estradiol benzoate and 10 days for estradiol. During this short period the normal metabolic system controlling inactivation of the naturally occurring estrogens probably is overwhelmed by the sudden introduction of relatively huge amounts of estrogen.

SUMMARY

Pellets of estradiol and estradiol benzoate implanted in the subcutaneous tissues of adult castrate female rats produce constant estrus for long periods.

Pellets of estradiol and estradiol benzoate implanted in the normally situated spleens of adult castrate female rats produce estrus for a short period, after which they are no longer able to exert their specific action.

When the pellet is in the spleen, the specific action of the estrogen can be demonstrated by diverting the blood from the spleen directly into the systemic circulation.

Estradiol and estradiol benzoate are inactivated in vivo in a manner similar to estrone.

The technical assistance of Ruth Helmuth is gratefully acknowledged.

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CHRONIC TOXICITY STUDIES OF DIETHYLSTILBESTROL

I. ORAL ADMINISTRATION BY STOMACH TUBE TO RATS

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THIS STUDY was made because of the conflicting reports in the literature regarding the toxicity of stilbestrol in man and animals. In many of these reports there is no evidence of controlled studies. The doses of stilbestrol are frequently excessive and so far above the calculated therapeutic dose range that the pathological changes reported are of little significance when considering the drug for clinical use. For these reasons this chronic toxicity study of the effects of stilbestrol in various dose ranges and in two different media was conducted.

METHOD OF STUDY

Four oral dose levels of stilbestrol were established for this study on rats as follows.

(*a*) Fifty γ 3 times weekly (750γ weekly/kg. body weight of rat) which is more than 5 times as great as the average human therapeutic dose (1 mg. daily for a 50 kg. person) or corresponds to a weekly dose of 37.5 mg. of stilbestrol for the human being.

(*b*) Two hundred and fifty γ stilbestrol 3 times weekly in rats (3.75 mg. weekly /kg.) which is more than 25 times as great as the average human therapeutic dose or corresponds to a weekly dose of 187.5 mg. of stilbestrol for the human being.

(*c*) One mg. stilbestrol 3 times weekly in rats (15 mg. weekly/kg.) which is about 107 times as great as the average human therapeutic dose or corresponds to a weekly dose of 750 mg. of stilbestrol for the human being.

(*d*) Five mg. stilbestrol 3 times weekly in rats (75 mg. weekly/kg.) which is more than 500 times as great as the average human therapeutic dose or corresponds to a weekly dose of 3,750 mg. of stilbestrol for the human being.

The rats used in these experiments were of Wistar Stock inbred for many generations. Normal albino females and males, and castrated females were used. The diet used for the rats was Purina Dog Chow which has maintained the colony in good health, vigor and fertility.

The crystalline stilbestrol used throughout was chemically pure.[1] As stilbestrol is not water-soluble it was dissolved in 40% ethyl alcohol or sodium hydroxide solution, prepared fresh before each administration. The sodium hydroxide solution was made as follows: the total amount of stilbestrol that was to be used for a group of rats at one administration was dissolved in normal NaOH solution (0.5 cc. N NaOH for each 20 mg. stilbestrol). This was then diluted with distilled water until the number of cubic centimeters of fluid was equal to the doses to be given, e.g., 20 animals

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[1] Supplied by Burroughs Wellcome & Co., New York.

TABLE 1. NORMAL FEMALE RATS

No. animals in each group	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5		
	5	20	25	5	12		
Menstruum	Dilute NaOH	Dilute NaOH	Dilute NaOH	1 cc. 40% E+OH	40% ethyl alcohol		
Dose stilbestrol 3X week	Control	50γ (0.05 mg.)	250γ (0.25 mg.)	Control	250γ (0.25 mg.)		
Duration of study, days	93	92	84	41	46	77	100
Total dose of stilbestrol, mg.	0	1.95	9	0	5	8.25	10.5
No. animals used for histological studies	1	4	4	2	2	2	2
Av. wt. at beginning of experiment, gm.	187	200	184	285	193	191.5	184
Av. wt. at end of experiment, gm.	210	200	195	284	135	214	160
% change in weight	+12	0	+8.1	−0.3	−30	+11	−13
Liver — Microscopic	Normal	Normal	Normal		Normal	Normal	Normal
Liver — Weight,[1] gm.		7.72; 3.94; 3.79	9.35; 4.79; 3.79				
Kidney — Microscopic	Normal	3 Normal 1 Cloudy swelling	2 Normal 2 Cloudy swelling		Normal	1 Cloudy swelling 1 Normal	1 Cloudy swelling 1 Normal
Kidney — Weight,[1] gm.		1.61; 0.81; 0.80	1.50; 0.77; 0.80				1.73; 0.81; 0.80
Spleen — Microscopic	Normal	Normal	Normal		Normal	Normal	Normal
Spleen — Weight,[1] mg.		906; 455; 566	518; 266; 566				
Adrenals — Microscopic	Normal	Normal	Normal		1 Hemorrhagic area in cortex; 1 Normal	Normal	Normal
Adrenals — Weight,[1] mg.	50.3; 24.0; 31.6	46.7; 23.4; 31.6	49.4; 25.3; 31.6		42.5; 31.5; 31.6	59.4; 27.7; 31.6	52.8; 33; 31.6
Pituitary — Microscopic	Normal	Normal	Hyperplasia ant. lobe		Hyperplasia ant. lobe	Hyperplasia ant. lobe	Hyperplasia ant. lobe
Pituitary — Weight,[1] mg.	12.6; 6.0; 5.3	12.9; 6.45; 5.3	13.3; 6.8; 5.3		16; 11.8; 5.3	16.2; 7.5; 5.3	11.1; 6.9; 5.3
Stomach	Normal	Normal	Normal	Normal	1 Atrophy of mucosa 1 Normal	Normal	Normal
Esophagus	Normal	Normal	Normal	Normal	1 Normal 1 Traumatic ulcer	Normal	Normal
Thymus — Microscopic	Normal		Normal		Normal		Normal
Thymus — Weight,[1] mg.			193; 98.9; 206				
Uterus — Microscopic	Normal	Endometrial hyperplasia	Endometrial hyperplasia	Normal	1 Normal 1 Endometrial hyperplasia	Endometrial hyperplasia	Endometrial hyperplasia
Uterus — Weight,[1] mg.	829; 395; 217	566; 288; 217	563; 289; 217			803; 375; 217	266; 166; 217
Vagina			Thickened epithelium	Normal			
Mammary gland	Normal		Hypertrophy of glands, cystic dilatation of ducts			Hyperplasia of ducts with cyst formation	
Ovary — Microscopic	Normal	Atrophy	Marked atrophy		Marked atrophy	Marked atrophy	Marked atrophy
Ovary — Weight,[1] mg.	49.1; 23.3; 33.1	13.1; 6.6; 33.1	15.4; 7.9; 33.1		11.2; 8.5; 33.1	18.7; 8.7; 33.1	15.8; 9.8; 33.1

[1] The organ weights from left to right are: actual weight, weight per 100 gm. body weight, control figures obtained from table 2 of Organ Weight Study by Emery, Emery, and Schwabe (8) on same rat colony.

to be given 1 mg. each, then 20 mg. of stilbestrol was dissolved in 0.5 cc. normal NaOH and diluted to 20 cc. with distilled water.

All of the stilbestrol in these experiments was given by stomach tube (the details of the method will be described in a separate paper). The stilbestrol was given to each group of animals 3 times weekly. All animals were weighed every 5 days and vaginal smears on the females were examined and recorded daily (the results of these data will be reported in a separate paper). At the end of the experiment the animals were killed and a complete autopsy was performed on each. If any tissue or organ appeared abnormal it was saved for microscopic study. In each group vital organs and endocrine glands of several animals were saved, regardless of gross appearance, for complete histologic study, and organs were weighed. In addition bone marrow and peripheral blood smears were made on most of the animals. Ten normal animals were autopsied and tissues were studied histologically so that comparisons between the treated and untreated animals could be made for each tissue studied.

RESULTS OF STUDY

1. *Normal Female Rats*

Sodium hydroxide controls. One group of 5 animals received 1 cc. 1/200 N sodium hydroxide solution by stomach tube 3 times weekly over a period of 93 days (*Group* 1, table 1). These animals served as the sodium hydroxide control group. The sodium hydroxide solution was about the normality of the solute in which stilbestrol was dissolved for the groups that received stilbestrol in NaOH. The average weight gain was 12%. Histologic study of vital organs (liver, kidney, spleen, stomach, esophagus), endocrine glands (adrenals, pituitary, thymus, ovary), and other organs (uterus, mammary gland), showed no abnormalities and the individual organ weights per 100 gm. of body weight were within normal limits (8).

Alcohol controls. A group of 5 animals received 1 cc. 40% ethyl alcohol by stomach tube every 3 days for 41 days (*Group* 4, table 1). These animals failed to gain weight. The esophagus, stomach and endocrine glands showed no abnormalities on histologic examination.

Stilbestrol (50γ) in NaOH solution. Twenty animals received 50γ stilbestrol in a dilute sodium hydroxide solution by stomach tube 3 times weekly (750γ weekly/kg.) for 92 days (*Group* 2, table 1). The total dose of stilbestrol for each animal was 1.95 mg. The animals failed to gain weight. At autopsy no gross pathologic lesions were found. Four of these animals were studied histologically. None of the vital organs or glands showed any significant change in weight except the ovaries which were about one-fifth of the average normal weight. Histologic examination showed no significant abnormalities in vital organs or glands except cloudy swelling in the kidney of one animal. The only histologic differences between this group and the control group were marked atrophy of the ovaries and moderate hyperplasia of the endometrium.

Stilbestrol (250γ) in NaOH solution. Twenty-five animals received 250γ stilbestrol in dilute sodium hydroxide by stomach tube 3 times weekly (3.75 mg. weekly/kg.) for a period of 84 days (*Group* 3, table 1). Each animal received a total of 9 mg. of stilbestrol. The average weight gain during the period of study was 8.1%. At autopsy none of the organs or glands showed gross pathologic changes or any significant change in weight except the ovaries which were about one-fourth of the average weight of normal ovaries and the thymus which was about one-half the weight of the average normal thymus. Four animals were studied histologically and it was found that none of the tissues or organs showed any abnormalities except cloudy swelling of the kidneys of 2 animals. Histologic variation from the normal was as follows:

TABLE 2. CASTRATE FEMALE RATS

		GROUP 1	GROUP 2				GROUP 3				GROUP 4
No. animals in each group		12	10				6				5
Menstruum		Dilute NaOH	Dilute NaOH				Dilute NaOH				40% E+OH
Dose stilbestrol 3× week		2507 (0.25 mg.)	1 mg.				5 mg.				5 mg.
Duration of study, days		51	14	21	28	42	14	21	28	42	49
Total dose of stilbestrol, mg.		5.25	6	9	12	18	30	45	60	90	105
No. animals used for histological studies		2	2	1	1	1	1	1	1	1	1
Av. wt. at beginning of experiment, gm.		234	163	180	207	184	154	136	196	218	..
Av. wt. at end of experiment, gm.		219	151	184	187	184	147	136	183	183	
% change in weight		−6	−7	+2	−9	0	−4	0	−6.6	−16	
Liver	Microscopic	Normal	Normal	Early toxic necrosis	Normal	Early fatty degeneration	Areas of focal necrosis	Areas of focal necrosis	Normal	Fatty degeneration of central lobule region	Normal
	Weight,[1] gm.	10.59; 4.84; 3.79								10.08; 5.51; 3.79	
Kidney	Microscopic	Normal	Cloudy swelling	Cloudy swelling	Normal	Normal	Normal	Normal	Normal	Cloudy swelling	Cloudy Swelling
	Weight,[1] gm.	1.48; 0.68; 0.80								1.58; 0.86; 0.80	
Spleen	Microscopic	Normal	Normal		Normal	Normal	Normal	Normal	Normal	Normal	Normal
	Weight,[1] mg.									743; 406; 566	
Adrenals	Microscopic	Normal	Normal	Normal	Normal	Normal	Early cortical cell necrosis	Areas of hemorrhage in cortex	Normal	Normal	Early cortical cell necrosis
	Weight,[1] mg.	54.6; 24.9; 31.6		62.4; 33.9; 31.6	71.5; 38.2; 31.6	82.5; 44.8; 31.6		54.8; 40.3; 31.6	57.7; 31.4; 31.6	67.0; 36.6; 31.6	

TABLE 2 (*Continued*)

No. animals in each group		GROUP 1	GROUP 2				GROUP 3				GROUP 4
		12	10				6				5
Pituitary	Microscopic	Hyperplasia ant. lobe	Hyperplasia ant. lobe	Hyperplasia ant. lobe	Hyperplasia ant. lobe	Marked hyperplasia ant. lobe	Marked hyperplasia ant. lobe	Hyperplasia ant. lobe	Normal	Marked hyperplasia ant. lobe	Marked hyperplasia ant. lobe
	Weight,[1] mg.	14.5; 6.6; 5.3	15.6; 10.3; 5.3	14.3; 7.7; 5.3	14.3; 7.6; 5.3	18.6; 10.1; 5.3	19.7; 13.4; 5.3	15.2; 11.1; 5.3	13.2; 6.7; 5.3	29.9; 16.3; 5.3	
Stomach		Normal	Normal	Normal	Normal	Normal	Low grade gastritis	Normal	Normal	Normal	Normal
Esophagus		Normal	Normal	Normal	Normal	Normal	Normal	Normal		Normal	Normal
Thymus	Microscopic	Normal								Normal	
	Weight,[1] mg.	373; 170; 206								183; 100; 206	
Uterus	Microscopic	Endometrial hyperplasia	Endometrial hyperplasia	Endometrial hyperplasia	Endometrial hyperplasia	Endometrial hyperplasia	Endometrial hyperplasia	Marked endometrial hyperplasia	Squamous endometrial metaplasia	Moderate endometrial hyperplasia	Endometrial hyperplasia
	Weight,[1] mg.	349; 156; 217	310; 205; 217		307; 164; 217	181; 98; 217	456; 310; 217		346; 189; 217	226; 123; 217	
Vagina		Increased thickness of epithelial tissue			Thickened epithelium			Thickening of epithelial tissue		Marked increase in thickness of epithelial tissue	
Mammary gland		Generalized adenomatosis				Cystic dilatation of ducts			Cystic dilatation of ducts		
Thyroid	Microscopic									Normal	
	Weight,[1] mg.									11.2; 6.6; 7.1	

[1] The organ weights from left to right are: actual weight, weight per 100 gm. body weight, control figures obtained from table 2 of Organ Weight Study by Emery, Emery, and Schwabe (8) on same rat colony.

marked atrophy of the ovaries, hyperplasia of the mammary glands, moderate hyperplasia of the endometrium and moderate hyperplasia of the anterior lobe of the pituitary gland.

Stilbestrol (250γ) in 40% alcohol. Twelve animals received 250γ stilbestrol in 40% ethyl alcohol by stomach tube 3 times weekly (*Group* 5, table 1). These animals were divided in 3 groups of 4 each and killed after 46, 77, and 100 days, respectively. The total dose of stilbestrol for each of these 3 groups was 5 mg., 8.25 mg., and 10.5 mg., respectively. The group as a whole showed a weight loss of 10.3%. At autopsy it was seen that one animal had had a hemorrhage into the adrenals, one had had a hemorrhage into the stomach and another had an ulcer of the esophagus. The individual organs showed no significant changes in weight except for a marked atrophy of the ovaries which were one-fourth the average weight of normal ovaries. Histologic examination of 6 animals (2 in each group) showed cloudy swelling of kidneys of 2 animals, adrenal cortical hemorrhage in one animal, moderate hyperplasia of the endometrium and of the anterior lobe of the pituitary gland in all animals. All mammary glands showed hyperplasia of ducts with cyst formation. Atrophy of the mucosa of the stomach was found in one animal and another animal had a traumatic ulcer of the esophagus.

2. *Castrate Female Rats*

Stilbestrol (250γ) in NaOH solution. Twelve animals received 250γ of stilbestrol in dilute NaOH by stomach tube 3 times weekly for a period of 51 days (*Group* 1, table 2). Each animal received a total of 5.25 mg. of stilbestrol. The animals lost an average of 6% in weight during the study. At autopsy none of the organs showed pathologic changes or any significant changes in weight from the normals. Two animals were studied histologically and none of the vital organs showed any pathologic changes. Endocrine glands and other organs showed moderate hyperplasia of the anterior lobe of pituitary gland, hyperplasia of the endometrium, and generalized adenomatosis of the mammary glands.

Stilbestrol (1 mg.) in NaOH solution. Ten animals received 1 mg. stilbestrol by stomach tube 3 times weekly for a period of 14, 21, 28 and 42 days (*Group* 2, table 2). These groups received during the period of study 6, 9, 12, and 18 mg. of stilbestrol, respectively. The weight change in these 4 groups were −7, +2, −9 and 0%, respectively. At autopsy no pathologic changes were seen grossly. In the 14- and 42-day groups the pituitary gland weight was about twice normal weight. Five animals were studied histologically. One animal showed early toxic degeneration of the liver and another showed early fatty degeneration of the liver. Kidneys of 3 animals showed cloudy swelling. Hyperplasia of the anterior lobe of the pituitary and hyperplasia of the endometrium was evident in all animals.

Stilbestrol (5 mg.) in NaOH solution. Six animals received 5 mg. stilbestrol by stomach tube 3 times weekly for periods of 14, 21, 28 and 42 days (*Group* 3, table 2). These animals received a total of 30, 45, 60 and 90 mg. stilbestrol, respectively. The weight changes during the period of study were −4, 0, −6.6 and −16%, respectively. At autopsy the adrenals of 3 animals appeared hemorrhagic; the gastric mucosa was hemorrhagic in one animal. The liver of the animal that received 90 mg. of stilbestrol was found to be increased about 50% in weight over that of normals. The pituitary glands in general were increased in weight up to three times normal in the animal that received 90 mg. stilbestrol; in this latter animal the thymus was about one-half the weight of the normal gland. Four animals were studied histologically. Livers of 2 animals (14- and 21-day animals) showed focal areas of necrosis and one showed fatty degeneration of central lobule region (42-day animal). The liver of

the other animal was microscopically essentially normal. Kidneys were normal in all but the 42-day animal which showed cloudy swelling. Adrenals showed early necrosis of cortical cells in one animal (14-day) and areas of hemorrhage in cortex of another (21-day) while the adrenals of the 28- and 42-day animals were entirely normal. The stomach of one animal (14-day) showed a low grade gastritis while the gastrointestinal tracts of the other animals were normal. All of the animals showed a marked hyperplasia of the anterior lobe of the pituitary and hyperplasia of the endometrium with one animal (28-day) showing squamous metaplasia of the endometrium.

Stilbestrol (5 mg.) in 40% alcohol. Five castrate female rats received 5 mg. in 40% ethyl alcohol by stomach tube 3 times weekly for 49 days (*Group 4*, table 2). The total dose of stilbestrol received by each animal was 105 mg. At autopsy no gross pathologic lesions were found. Body and organ weights were not obtained for animals of this group. One animal was studied histologically. The liver was normal. The kidney showed cloudy swelling. There was fatty infiltration of the cells of the adrenal cortex. Marked hyperplasia of the anterior lobe of the pituitary and of the endometrium was present.

3. *Normal Male Rats*

Twenty animals received 1 mg. stilbestrol in dilute NaOH by stomach tube 3 times weekly for 34 days, receiving during that time 11 mg. of stilbestrol (table 3). During the course of study the animals gained 6% in weight. Two of these animals were studied histologically. The pituitary gland showed hyperplasia of the anterior lobe. The testes were markedly atrophied weighing about one-third of the normal. The cells in the spermatic tubules were thinned out with no spermia evident and spermatids were markedly reduced in number. Primary spermatogonia were the most prominent cells in the tubules. The interstitial cells also were greatly thinned out so that only an occasional island of cells was present. The prostate showed hyperplasia of the glands. The seminal vesicles showed a generalized chronic inflammatory reaction.

Blood Studies

Careful study of peripheral blood and bone marrow smears did not show any evidence of injury to the blood forming organs until the dose received was 15 mg. weekly. Two animals receiving this large dose showed hypoplasia of the bone marrow with leucopenia, thrombocytopenia, neutropenia, achromia and polychromatophilia.

DISCUSSION

In this study on the chronic toxicity of stilbestrol by the oral route, 130 animals were used. One hundred and ten of them received stilbestrol in varying doses; 10 treatment controls received only 40% ethyl alcohol or a dilute solution of sodium hydroxide, and 10 were normal animals used for comparative histology. An autopsy was performed on all animals with careful inspection of their tissues and organs, with body and organ weights recorded. Peripheral blood and bone marrow smears were made on most of the animals. In the autopsy protocols there was recorded a description of any gross anatomic lesions that were encountered and histological preparations were made. In all groups regardless of gross findings representative animals were selected for complete histologic study. The tissues of 41 animals were studied microscopically.

The changes produced in these animals by stilbestrol may be divided into physiologic effects of the drug and pathologic changes.

Physiologic effects of stilbestrol. The most consistent physiologic effect on female rats was ovarian atrophy which caused 70 to 80% decrease in weight of the organ. His-

tologically there was a marked reduction in number and size of the follicles with a relative increase in the stroma. The pituitary showed mild hyperplasia when smaller doses of stilbestrol were administered. When the dose ranged from 1 to 5 mg. three times a week there was an increase in the weight of the gland from 40 to 200%. Histologically all of the sections showed hyperplasia of the anterior lobe.

Grossly many of the uteri were distended with fluid and cheesy material, and histologically there was endometrial hyperplasia. There was an increase in the thickness

TABLE 3. NORMAL MALE RATS

No. of animals		20
Menstruum		Dilute NaOH
Dose stilbestrol 3×weekly, mg.		1
Duration of study, days		34
Total dose of stilbestrol, mg.		11
No. animals used for histological studies		2
Av. wt. at beginning of experiment, gm.		183
Av. wt. at end of experiment, gm.		195
% change in weight		+6
Pituitary	Microscopic	Ant. lobe hyperplasia
	Weight,[1] mg.	10.6; 5.4; 3.2
Testes	Microscopic	Marked atrophy
	Weight,[1] mg.	729.1; 373.9; 1147.0
Prostate microscopic		Glandular hyperplasia
Seminal vesicles	Microscopic	Inflammatory reaction and congestion
	Weight,[1] mg.	60.7; 31.1
Stomach		Normal

[1] The organ weights from left to right are: actual weight, weight per 100 gm. body weight, control figures obtained from table 3 of Organ Weight Study by Emery, Emery, and Schwabe (8) on same rat colony.

of the vaginal epithelium. The ends of the uterine horns were enlarged and congested in many animals.

The mammary gland showed marked glandular hyperplasia and in many instances there was in addition cystic dilatation of the ducts, some of which contained retained secretions.

In those animals on which thymus weights were taken there was a considerable reduction in weight. Stilbestrol in male rats produced marked atrophy of all of the cellular elements of the testes and there was a 70% reduction in the weight of these organs.

Pathological effects of stilbestrol. Of the animals that received 50γ of stilbestrol 3 times weekly the only histological lesion found was cloudy swelling of the kidney of one animal of the 4 studied. No gross pathologic lesions were found at autopsy of any of the 20 animals that received this dose of the drug.

Of the animals that received 250γ stilbestrol 3 times weekly, cloudy swelling was found in 4 kidneys of the 12 animals studied histologically. Hemorrhagic areas were found in the cortex of the adrenal of one animal out of 49 animals autopsied.

The animals that received 1 mg. of stilbestrol 3 times weekly showed cloudy swelling of the kidneys of 6 of the 8 animals studied. In addition the livers of 4 animals showed early fatty degeneration, one liver showed early toxic necrosis while the livers of the remaining 3 animals were entirely normal.

The animals that received 5 mg. stilbestrol 3 times weekly showed cloudy swelling of the kidneys of 2 of the 5 animals studied histologically. The livers showed focal necrosis in 2 animals, fatty degeneration in one animal and were normal in the remaining 2 animals. The adrenals grossly showed moderate hyperplasia (table 2) and histologically showed early cortical cell necrosis in 2 animals, areas of hemorrhage in cortex of one animal, and the adrenals of the remaining 2 animals were normal. One animal showed a low grade gastritis.

In general the animals that received stilbestrol in 40% alcohol ate poorly and lost considerably more weight than the animals in the corresponding sodium hydroxide group.

It is difficult to draw conclusions from the reports of Loeser (1, 2) because they lack data relating to the method of study, number of animals used, and whether control animals were studied. He reported subconjunctival and nasal hemorrhage but the number of animals showing these phenomena was not stated. No studies of the blood were reported and therefore the bleeding cannot be ascribed to injury to the blood-forming organs by stilbestrol.

Mazer (3) stated that 0.1 mg. stilbestrol daily for 42 days produced congestion of the vital organs and that 1 mg. daily produced definite necrotic changes in the liver, spleen and kidney. We found no abnormal congestion in any of the animals receiving stilbestrol. No necrosis of the spleen or kidney was found in any of our animals regardless of dose or length of time stilbestrol was given.

The local irritating effect of stilbestrol that has been described by some authors (4, 5) was found to be present in only 2 of our animals; one had an atrophy of the gastric mucosa (*Group* 5, table 1) and the other had a low grade gastritis (*Group* 3, table 2). Our findings suggest that in the rats used in this study the large doses of stilbestrol caused only a very low incidence of local irritation of the gastrointestinal tract. One other animal had a traumatic ulcer of the esophagus (*Group* 5, table 1) which can probably be ascribed to mechanical irritation caused by the passing of the stomach tube. We believe that our stomach tubing technic is a relatively safe procedure for rats as we found only one esophageal lesion although many of the animals were tubed as much as 33 times.

Our findings in regard to blood-forming organs agree essentially with those reported by MacBryde et al. (6) and Arnold (7). Inasmuch as no detailed figures were presented by these observers on the changes in the blood it is difficult to make comparisons. Our studies indicate that injury to the blood-forming organs does not take place until doses (15 mg. weekly to rats) of stilbestrol are given that are approximately 500 times the average human therapeutic dose. Then the injury appears to cause a hypoplasia of the marrow with a depression of all the cellular elements of the peripheral blood. We would interpret this as indicating aplastic anemia. In view of our findings of injury to the blood-forming organs following massive doses of stilbestrol we will make a more detailed study of this effect of the drug which will be reported at a later date.

There was no gross nor histologic evidence of tumor formation in any of the animals.

Three of the ten normal rats studied showed cloudy swelling of the kidneys on microscopic examination. We attribute this to postmortem changes that occurred before fixation took place. We therefore feel that our findings of cloudy swelling of some of the kidneys of the animals that received stilbestrol cannot entirely be attributed to the drug.

SUMMARY

No gross pathologic lesions were observed in the rats that received 750γ/kg. weekly for 14 weeks (approximately 5 times the average human therapeutic dose). No pathologic changes were found on microscopic examination except for a single instance of cloudy swelling of the kidney.

Gross pathologic lesions observed at autopsy in the 49 rats that received 3.75 mg./kg. weekly for 7 to 14 weeks (approximately 26 times the average human therapeutic dose) were hemorrhagic gastric mucosa in one animal, traumatic ulcer of the esophagus in one animal and hemorrhage into the adrenals of one animal. Pathologic changes on microscopic examination were limited to cloudy swelling of the kidneys of 4 animals.

No gross pathologic lesions were observed in the rats that received 15 mg./kg. weekly for 2 to 6 weeks (approximately 107 times the average human therapeutic dose). Microscopic examination showed pathologic changes consisting of cloudy swelling of the kidneys of 3 animals, and liver damage in 2 animals.

Gross pathologic changes at autopsy of the 11 animals that received 75 mg./kg. weekly for 2 to 7 weeks (approximately 535 times the average human therapeutic dose) were adrenal hemorrhage in 3 animals and enlarged liver in one animal. Microscopic examination showed the following: liver damage in 3 animals, cloudy swelling of kidneys of 2 animals, adrenals of 3 animals showed toxic changes, gastritis was present in one animal, squamous metaplasia of the endometrium was present in one animal and blood studies of 2 animals showed hypoplasia of the bone marrow, leucopenia, neutropenia, thrombocytopenia, achromia and polychromatophilia. We interpret these blood findings as aplastic anemia.

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EARLY ESTRUS IN THE CAT FOLLOWING INCREASED ILLUMINATION

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THE BREEDING CYCLE of the cat is characterized by a definite anestrous period. The data of Foster and Hisaw (1) indicate that in the region of Madison, Wisconsin, cats are usually anestrous from September to January although a few may be found in this phase of the cycle at still later dates. Extensive observations by Windle (2) on several hundred cats over a period of 11 years show that estrus rarely occurs (under laboratory conditions in Chicago, Illinois) during the season beginning with July and ending with January. Accordingly, Windle regards the period from February to June, inclusive, as the normal estrous season.

Experience over the past 3 years in Cambridge, Massachusetts also confirms these observations on the duration of the anestrous period. Few animals exhibited spontaneous estrus in the early part of January (table 1). Many investigators have induced estrus during the anestrous period by the injection of appropriate hormones. The greatest measure of success has been obtained by using FSH. This treatment supplemented by LH without the stimulation of mating may also result in ovulation. However, during the anestrous period, only limited success has followed treatments with PU or PS (2). The explanation of this seasonal refractoriness is not known.

TABLE 1. DATES OF FIRST ESTRUS OF CATS UNDER OBSERVATION DURING THE EARLY BREEDING SEASON OF 1938, 1939 AND 1940. THE RECORD FOR AN INDIVIDUAL CAT IS INDICATED BY THE CALENDAR YEAR IN WHICH THE OBSERVATION WAS MADE

January 1—'39	February 21—'39, '40
January 9—'38	February 23—'40
January 13—'39, '39	February 24—'39, '39, '38
January 21—'39, '39	February 25—'39
January 23—'39	February 27—'39
January 25—'39	February 28—'38, '39
January 28—'39	March 2—'39, '39, '39
January 29—'39	March 3—'38
January 30—'39	March 5—'39
January 31—'38	March 6—'39, '39
February 1—'39	March 7—'39
February 2—'39	March 9—'38, '39
February 3—'39, '39	March 14—'38
February 8—'40	March 15—'38
February 11—'39, '39	March 17—'39
February 12—'39	March 21—'39
February 13—'40	March 24—'38, '38
February 15—'39	March 27—'39
February 16—'39, '39	March 30—'38
February 17—'39	April 1—'38
	April 10—'38

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The present interest in the cytological changes in the anterior pituitary gland during the various phases of the reproductive cycle precluded the induction of estrus by the use of hormones. It was felt that these treatments might produce atypical cellular changes which would obscure the normal picture of glandular activity. Accordingly, if the period of breeding activity of the cat were to be extended or modified, it was necessary to find some more natural means of bringing about early estrus.

The most obvious factor of the environment which may be readily changed is the period of daily illumination (3, 4). The onset of the period of anestrus is closely related to the decreasing length of day. Cats usually cease breeding actively 3 to 5 weeks after the longest day of the year. Estrus is relatively common in June and becomes rare during July, the latest dates recorded for our colony being July 11, 16 and 17, although animals were observed daily until late in August. Correspondingly, the onset of the breeding period occurs 3 to 5 weeks after the shortest day of the year, that is, under an increasing daily increment of light.

In January 1938, cats were subjected to increased illumination with the hope that under these conditions the onset of estrus during the normal breeding season might

TABLE 2. DATA ON 4 CATS WHICH EXHIBITED EARLY ESTRUS FOLLOWING INCREASED ILLUMINATION

No. of animal	Period of increasing illumination, days	Date of estrus	Mated—cervical stimulation	Period before autopsy	Observations
58	49	12/13/39	Mated twice; Stimulated twice	48 hr.	Right ovary—2 ovulation points Left Ovary—2 ovulation points
59	56	12/20/39	Mated twice	29 hr.	Right ovary—2 ovulation points Left ovary—2 ovulation points
60	61	12/25/39	Mated twice	8 days	Right ovary—2 corpora lutea Left ovary—1 corpus luteum; 1 cystic follicle
61	62	12/26/39	Refused male; Stimulated twice	6 days	Right ovary—2 corpora lutea Left ovary—1 corpus luteum

be hastened. These experiments were terminated after three weeks since there was little evidence of an increase in the number of animals coming into estrus.

In the autumn of 1939 increased illumination was again employed. Seven cats which had been under daily observation since the preceding spring were exposed to gradually increasing periods of lighting. These cats had not exhibited estrus while in the laboratory. The animals were kept at a temperature of 71° to 73° F. in an air-conditioned room 18 feet long, 12 feet wide and 8 feet 9 inches high. The room had a southern exposure on the fifth floor of the laboratory with a window 4 feet 6 inches high and 4 feet wide. The lighting consisted of two 200-watt clear bulbs suspended from the ceiling. The lights were turned on and off by a time switch for the following hours each day: Oct. 25 to Nov. 7, 4 to 5:30 P.M.; Nov. 8 to 14, 4 to 7:30 P.M.; Nov. 15 to 21, 3:45 to 8:30 P.M.; Nov. 22 to Feb. 1, 3:45 to 9:30 P.M. The results of this experiment are given in table 2. Four of the seven animals exhibited early estrus. The other 3 proved refractory and never came into heat spontaneously. In May they were injected with estradiol and at autopsy their reproductive tracts were found to be normal with the ovaries present.

The lighting experiments were repeated in 1940. The number of cats was limited to 6 in order that they might receive special attention and be maintained in optimum physical condition. Preceding the period of increased illumination, the day was artificially shortened by shuttering all sources of light from 4:30 P.M. to 9:30 A.M. These

animals accordingly had 7 hours of natural illumination followed by 17 hours of darkness for all days during this period except Saturdays and Sundays when the shutters were left open. The cats were maintained under these conditions from August 22 to October 1, and then placed under increasing light according to the following schedule: Oct. 2 to 9, 4:30 to 5:30 P.M.; Oct. 10 to 16, 4:30 to 6:30 P.M.; Oct. 17 to 23, 4:30 to 7:30 P.M.; Oct. 24 to 30, 4:30 to 8:30 P.M.; Oct. 31 to Nov. 6, 4:30 to 9:30 P.M.; Nov. 7 to 9, 4:30 to 10:30 P.M. After November 10, the artificial illumination of 6 hours daily was maintained and in addition the shutters which previously had been closed until 9:30 A.M. were opened at 5 P.M. Accordingly, during this last period, the animals received 6 hours of artificial light together with the available morning light. All six animals exhibited early estrus. Since no male was available, an attempt was made to induce ovulation, usually successfully, by stimulation of the cervix uteri according to the method of Greulich (5). The history of these animals is shown in table 3.

TABLE 3. DATA ON 6 CATS WHICH EXHIBITED EARLY ESTRUS AFTER A PERIOD OF DECREASED ILLUMINATION FOLLOWED BY INCREASED ILLUMINATION

No. of animal	Period of decreased illumination	Period of increasing illumination	Date of estrus	Cervical stimulation	Period before autopsy	Observations
	days	*days*			*days*	
92	39	50	11/21/40	twice	4	No ovulation Right ovary—2 large follicles Left ovary—12 large cystic follicles
93	39	55	11/26/40	twice	3	Right ovary—1 ovulation point Left ovary—3 ovulation points
94	39	58	11/29/40	twice	4	Right ovary—5 ovulation points Left ovary—1 large follicle
95	39	63	12/ 4/40	twice	7	Right ovary—3 corpora lutea Left ovary—small follicles
96[1]	39	68	12/ 9/40	twice	12	Right ovary—3 ovulation points
		75	12/16/40	twice	5	Left ovary—4 ovulation points
97	39	71	12/12/40	twice	12	Right ovary—2 corpora lutea Left ovary—3 corpora lutea

[1] Gave typical coital response on Dec. 9 followed by marked estrous behavior on Dec. 16. Cervical stimulation on the latter date induced ovulation.

The ovaries of the cats used in the last experiment have not been studied histologically. The left ovary of *cat* 92 was abnormally large (15×12 mm.) resembling the ovaries of animals maximally stimulated with FSH. The right ovary was not so large (13×7 mm.). No explanation can be offered for the atypical behavior of *cat* 96. The reactions on Dec. 9 were those of typical estrus with a good response to mechanical stimulation. However, it had obviously ovulated following cervical stimulation on Dec. 16 since the ovulation points were still bright red. Both ovaries were large (right, 11×7 mm.; left, 10×8 mm.) and contained many medium-sized follicles. The ovaries of the other 4 cats did not appear different from those of animals autopsied during normal estrus.

The attempt to induce earlier estrus during the natural breeding season was apparently discontinued too soon, since, in the two succeeding experiments, the effects of increased lighting were initially obtained only after 49 and 50 days, respectively (see addendum). The data indicate a considerable variability as to the time of response, with a spread of 13 days in one group and 21 days in the second group. From the limited observations available it would appear that previous shortening of the day did not appreciably affect the response to increased illumination.

The data on the normal occurrence of estrus (table 1) indicate that it occurs infrequently before the middle of January. However, in one instance (not included in the table) a cat received at the laboratory on January 3, 1940 aborted the following day. From fetal size and weight it was calculated that the pregnancy was of at least 53 days duration, placing estrus on or near November 12. This is an exceptional record but, in this instance, nothing was known of the history of the animal prior to her delivery at the animal farm. The advance in the time of estrus of the 10 animals reported above seems significant and indicates that increased lighting is effective at least during the latter half of the period of anestrus. The extent to which the breeding cycle may be influenced by this single environmental factor is problematical. The need for further experimental evidence is obvious.

SUMMARY

In the latitude which includes Cambridge, Mass., cats have a normal breeding period usually extending from the middle of January to the middle of July. In two experiments conducted in the autumn of 1939 and 1940, early estrus was induced by increased illumination during the anestrous period. In 1939, 4 out of 7 cats responded. Lighting experiments were begun on October 25; the first estrus was obtained 49 days later, on December 13, with the last cat exhibiting estrus 13 days later.

In 1940, six cats were used. They were subjected to decreased illumination for 39 days before receiving the additional lighting. Lighting was begun on October 2; all 6 cats responded. The first animal was observed in estrus on November 21 after 50 days of increased lighting and the last on December 12. Previous shortening of the day apparently did not reduce the time before the response occurred. Although a small number of animals was used, the results are regarded as significant. The intervals of increased lighting preceding the first response were the same in each experiment although illumination was begun three weeks earlier in the anestrous period of 1940.

ADDENDUM

More recent observations suggest that increased illumination will not accelerate the onset of estrus during or close to the normal breeding season. Animals brought into the laboratory December 23, 1940 and subjected immediately to weekly increments of lighting, as in the 2 experiments conducted during anestrus, exhibited first estrus at rather widely spaced intervals—37, 43, 45, 60 and 100 days, respectively, after the initiation of illumination. The time of estrus was apparently previously conditioned by the normal daily light increments received up to the last of December. The ovaries of 3 of these cats were markedly cystic and ovulation did not occur following mechanical stimulation of the cervix.

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A TECHNIC FOR ASSAY OF ESTROGEN BY EVALUATION OF HUMAN VAGINAL SMEARS AND COMPARISON WITH URINARY ESTROGEN ASSAY ON THE MOUSE UTERUS[1]

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Investigators and clinicians who have been using human vaginal smears either to control dosage of estrogen in the climacterium or to study the female sex cycle have in their reports implied the existence of a relation between the degree of proliferation in the vaginal mucosa as represented by the vaginal smear and the level of estrogen in the patient or subject. This implied relation was never itself investigated since the difficulties of collecting and assaying a large number of 24-hour urine specimens and vaginal smears seemed almost prohibitive and besides, there is no a priori reason why vaginal smears should correlate with the excreted estrogen or even with the circulating estrogen (blood methods being crude and inaccurate).

In the course of our investigation of the female sex cycle it became possible to collect an adequate number of urine specimens and smears of 5 patients, and to see whether there was indeed a correlation between smears and urinary estrogen which could be made explicit.

The technical details of vaginal smear collection, staining, and study presented in a previous publication (1), were followed. Naturally the precautions regarding taking of the smears *a*) from the posterior fornix of the vagina, *b*) in the absence of infection and irritation, and *c*) fixing the smears before they may have dried must be observed faithfully. Systematic deviations of the smear evaluation curves from the urinary estrogen estimation curves occur when coitus increases the proportion of cornified cells in the smear of the day or days following, although estrogen excretion is low. Each urine specimen and each vaginal smear was given an identifying mark by a third person. We treated them as unknowns. The 24-hour urines (unpreserved) were extracted with benzene and assayed on 10-day-old female mice (2).

The urine was acidified with hydrochloric acid to pH 2, using a Hellige pH meter as the measuring instrument; the acidified urine was boiled for 15 minutes, cooled, and placed in a counter-flow column extractor for subsequent thorough extraction with benzene. Each sample of urine was extracted with at least 15 volumes of benzene to make certain that all of the estrogenic material had been removed from the urine. The benzene extract was evaporated to dryness under reduced pressure, and the residue dissolved in alcohol, and made up to constant volume in a volumetric flask. A quantity of the alcoholic solution (0.5 cc.) equivalent to one-tenth of the 24-hour sample was added to 4.5 cc. of sesame oil. The oil-alcohol mixture was heated in a water bath under reduced pressure until all of the alcohol had been removed. The resulting oil solution containing both the estrogens and androgens from the urine was used for the assay.

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[2] Rockefeller Foundation Fellow.

The results reported in this study were obtained using the method of assay described in the paper of Evans, Varney and Koch (this journal, May issue) (2). All values are reported in terms of uterine weights as compared to uterine weights of control mice receiving known amounts of crystalline theelin in oil. The comparison of these results with those obtained by evaluation of the vaginal smears of the patients is presented below. This comparison was not made until after each of us had completed his part of the study.

The results obtained by the two methods indicate that the vaginal smears show a clear relation to estrogen levels during the normal cycle and when estrogen is given by injection.

To make the correlation, we started with the empiric observation that in childhood, after the menopause, and in occasional patients with non-functioning ovaries, the vaginal mucosa is thin and the smear consists of small round cells with relatively large vesicular nuclei (basal cells, type 8);[3] we had also the converse observation that in women receiving large doses of estrogens, and occasionally also in the mid-part of the normal cycle the vaginal mucosa is thick and the smear consists of plaques of squamous cells with hyalin cytoplasm and small, pyknotic or fragmented nuclei (type 4).[3] Smears consisting of type 8 cells were assumed to indicate zero estrogen production; smears consisting of type 4 cells were assumed to represent maximal estrogen response (estrogen IV). Intermediate smears were assigned intermediate estrogen values according to the following scheme: that the minimal response to estrogen during the normal cycle (cell types 6, 7, 1)[3] was called estrogen I; smears of the proliferative phase consisting of cell types 1 and 2[3] predominantly were classified as estrogen II; and smears of the preovulative or postovulative phase consisting chiefly of cell types 2 and 3, or 3 and 5[3] respectively were classified as estrogen III.

MATERIALS AND METHODS

Of the 5 patients studied two were classified as cases of primary amenorrhea and three of 'functional' sterility.

Case 1. A white female, age 33, had been married 7 years and had had 1 pregnancy, ectopic, 6 years before. Rubin's and Hühner's test results were normal. Preliminary study by vaginal smears-basal rectal temperature technic for 6 months showed one spontaneous ovulation, but also presumptive progesterone deficiency during the corpus luteum phase of that cycle. Urines and vaginal smears were collected daily from the beginning of one menstrual flow to the onset of the next menstrual flow. No treatment was given.

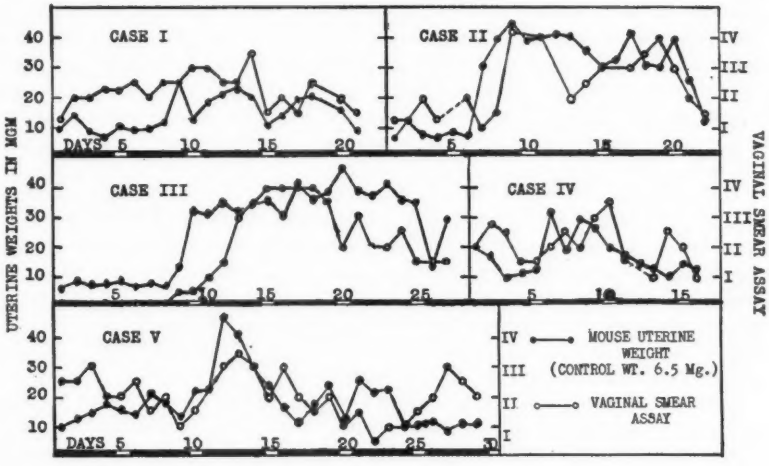
Case 2. A white female, married, was 36 years old. She is tall and slender, has grown 5 in. since the age of 21, and has lost 10 lb. in the past 5 years. She was referred because of her amenorrhea, frequent headaches, and generalized muscular pain of shifting character and variable intensity. Examination revealed absence of glandular tissue in the breasts which were perfectly flat, and with small nipples like those of a male. She had no pudendal hair. External genitalia were small like those of a 10-year-old girl. Pelvic examination revealed normal vagina, normal, firm cervix. The corpus of the uterus was small, not more than 1 in. in diameter and firm. Adnexa were not palpable. Treatment with equine gonadotrophin and with pregnancy urine extract aggravated her symptoms. She had had some benefit in previous years from occasional injections of estrogenic substance. She was put on the same experimental regime as *case 3*.

Case 3. A white female, 33 years old, was rather short and obese, unmarried, and had never menstruated. Pelvic examination revealed infantile uterus, ovaries not palpable, cervix barely distinguishable, vagina small, admitting only one finger. External genitalia were grossly normal. No clitoridal enlargement was found. This patient was referred because of nervousness, hot flashes, hirsutism, obesity, and primary amenorrhea, for study and treatment. Pre-

[3] The vaginal smear cell types are described in detail in a previous publication (1).

vious treatment with gonadotrophins over a period of a year using both equine and chorionic gonadotrophins either singly or together had produced only aggravation of her symptoms, and no change in her amenorrhea, obesity, and hirsutism. Since it seemed advisable to treat this patient with estrogen she was included in our study as a control. Twenty-four-hour urine specimens and daily vaginal smears were obtained for 27 consecutive days, the duration of our study. During the first 8 days no treatment was given. During the next 16 days 1.66 mg. estradiol benzoate in oil[4] was given intramuscularly daily. After 8 days of estradiol benzoate alone 10 mg. of progesterone in oil[4] was given daily for 5 days. The progesterone was then withdrawn but estradiol was continued until bleeding occurred 3 days later, at which time estradiol was also discontinued. Urine collections were continued until the flow ceased.

Case 4. A white, female, married, was 32 years old. This patient had had one child by a previous marriage 7 years ago. There had been no pregnancies during her second marriage.



Rubin's and Hühner's test results were both repeatedly positive. This patient was very obese, her weight having increased by 12 lb. during the past year. Her periods were very irregular, occurring every 50 to 70 days without pain or pre-menstrual discharge. The menstrual flow itself was profuse, and continued from 8 to 15 days. Previous studies of her cycle by the vaginal smear-basal body temperature technic during a period of one year had revealed no ovulations. During the period of this study she received one 40 U intravenous injection of equine gonadotrophin (gonadogen[5]) on the 6th day after ending the menstrual flow, that is on the 15th day of her cycle.

Case 5. A female, white, married was 35 years old, and had had no pregnancies. Previous study of the cycle revealed it to be essentially normal. Rubin's test and pelvic examination revealed no abnormalities; the Hühner's test result was repeatedly negative. Daily urine specimens, and vaginal smears were taken from the end of one menstrual flow through the next menstrual flow, for a period of 32 days. The patient omitted collection of 4 specimens during this month.

DATA AND DISCUSSION

Figure 1 presents in summary the data we obtained on the 5 patients. Each pair of curves represents the data of one patient. The heavy line curve represents the mouse uterine weight response to the estrogen extracted from 1/10 of that day's

[4] Supplied through the courtesy of Dr. Max Gilbert of the Schering Corporation, Bloomfield, N. J.
[5] Supplied through the courtesy of Dr. E. Gifford Upjohn, The Upjohn Company, Kalamazoo, Michigan.

urine specimen. The light line represents the estrogen assay by the human vaginal smear evaluation scheme presented above. It should be noted that since the control uterine weights were 6.5 mg., this value represents a zero response to estrogen. The calibration curves for urinary estrogen are presented in detail together with the technic in the publication by Evans, Varney and Koch (2). The differences between the curves suggest that the vaginal mucosa may be a little less responsive to estrogen than is the mouse uterus. The vaginal smears show a slight lag in rising and a somewhat faster fall than does the curve of uterine weight. It should be noted that there was in the smears at the end of the curve 4 and at both the beginning and end of curve 5 evidence of coitus (presence of sperm). The irritation in the vagina resulting from coitus apparently was sufficient on these occasions to lead to proliferation out of proportion to the then existing hormone. The discrepancy in smear evaluation after coitus should serve as a warning that smears in which there is evidence of coitus may give disproportionately high estrogen assay values. On the whole, however, there seem to be fairly clear-cut correlations between the urinary and vaginal smear estrogen assay values. Both curves show the same general pattern; and when the one gives indication of high estrogen values the other checks it. Thus when no precise assay is required, the human vaginal smear seems to afford a simple substitute on the scale of 5 (0–4).

The recent descriptions of day-by-day changes in vaginal smears during the menstrual cycle of normal women suggested the possibility that vaginal smears might also be used to assay estrogen secretion by the ovaries of the woman herself. While evaluation of vaginal smears at best permits only an approximation to the degree of vaginal proliferation and of estrogen levels, by inference; assay of urinary estrogen is also only an approximate technic. Any method which circumvents the necessity for extraction of hormone from the urine, and its subsequent bioassay will save both time and money, and may prove to be clinically useful.

The data presented indicate that the human vaginal smear may be used as an approximate or crude assay method for estrogen production. It indicates even more, namely that estrogen excretion is proportional to estrogen production and that consequently estimation of urinary estrogen provides a fair criterion of the gonad function. The results presented tend to confirm the current impression that estrogen production fluctuates fairly widely from day to day but that there exists a regular cycle in the normal woman upon which the daily fluctuations are superimposed. The general conclusion that for clinical purposes the vaginal smear should be the method of choice in estimating estrogen seems inescapable. The method obviates the necessity for expensive extraction and bioassay procedures substituting for them the relatively inespensive staining and microscopic examination of the vaginal smears, which can be made a simple office procedure.

SUMMARY

A technic of evaluation of human vaginal smears in terms of estrogen on a scale 0–4 is presented. The assay by human smears roughly parallels the assay of urinary estrogen excretion using the mouse uterus weight response. The human vaginal smears taken with proper precaution may be used as a rough assay of the patient's estrogen.

We are glad to acknowledge the interest and helpful advice of Dr. F. C. Koch.

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INTESTINAL ABSORPTION OF SUGARS IN THE TOAD WITH HYPOPHYSEAL OR ADRENAL INSUFFICIENCY

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THE TOAD *Bufo arenarum* Hensel, is the zoological species in which the greatest number of symptoms due to extirpation of the whole hypophysis or of only one of its two lobes have been observed. This animal reacts strongly and quickly to the implantation or injection of pituitary substances which can either prevent and correct the symptoms of insufficiency or produce manifestations of hyperfunction of the gland. Houssay (1) has summarized these studies.

Hypophyseal and Adrenal Asthenia

Asthenia is one of the most characteristic signs of hypophyseal insufficiency in this toad. The asthenia involves the locomotor system, smooth muscle organs and heart. It is apparently due to profound metabolic disturbances which lead to death in nearly all cases 2 to 6 weeks after removal of the hypophysis. The asthenia is also observed in other batrachians, but it is particularly characteristic in *Bufo arenarum* due to its severity and prolonged period of evolution (2–6).

The asthenia, diminution of activity, or hypodynamia is first observed 15 to 20 days after operation. In hot weather it can appear earlier (7 to 16 days) and at low temperatures 8–10° C.) it appears later. The evolution of the asthenia is characteristic. The first sign is an alteration of postural reflexes. When the animal is placed on its back, it does not turn over as quickly as does the normal animal; it remains immobile for some seconds with legs catatonically retracted or in bizarre positions. After a time, the animal moves its legs and eventually turns over. The more pronounced the asthenia, the more difficult it is for the animal to turn over. In severe cases, it may require 5 to 10 minutes for the normal posture to be assumed and terminally they may be unable to turn over. That there is not paralysis is proven by the fact that the animal can move its legs vigorously and rapidly without turning over. There is no cutaneous anesthesia since the electrical threshold is normal and the excitability to acids is frequently increased.

Little by little other asthenic signs are observed. In the resting state, the head is lower and the legs are more widely separated than in the controls. On holding the head of the animal between the thumb and index finger, the legs hang flaccidly and the animal struggles less than normally. In water, the toad sinks more easily. The singing reflex, induced by pressing the sides, persists nearly until death. Spontaneous movements decrease. The provoked march becomes quadrupedal instead of the customary jumps. Movements are infrequent, the muscles become somewhat rigid, and at the end the animal is immobile and without reflexes. Only the feeble heart beats

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show that it is still alive. At the beginning of the period of asthenia or during its evolution, convulsions are seen in a variable percentage of toads, in most groups 5 to 10% but exceptionally as high as 50%.

The development of asthenia is more rapid after complete extirpation of the hypophysis than after that of the pars glandularis only.[1]

The asthenia can be prevented or cured by implantation or injection of the anterior lobe of mammals or of the principal lobe of toads while the posterior lobe of mammals and neuro-intermediary lobe of toads are less active and often toxic. Glucose and epinephrin have no curative action (4, 5, 7, 8).

During asthenia, muscular and especially nervous alterations can be revealed. One of the first signs to appear is the alteration of postural reflexes. Generalized clonic-tonic convulsions are often observed. On studying the crossed reflexes of one leg by excitation of the central end of the sciatic nerve of the other side during hypophyseal asthenia, chronaxie is found to be increased, there is rapid fatigue of the reflex, and in more advanced cases the reflex disappears. The chronaxie of muscle is not changed except when pronounced asthenia is present (5). The curves of muscle fatigue are similar to or slightly lower than those of normal toads. A decrease in muscle glycogen (9), and of phosphocreatine and glutathione (10) has been observed.

Changes in the heart are as follows: bradycardia, increase of rheobase and chronaxie (5, 11), and decrease of the cardiomoderator excitability of the vagus (5). The blood pressure decreases and capillary and arteriolar dilatation occurs (12).

During hypophyseal and adrenal asthenia, dilatation, congestion, and decrease of motility of the stomach and intestine occur. The response to epinephrin and acetylcholine is diminished. Histologically dilatation of blood and lymph vessels and edema of the chorion especially in the submucous layer can be observed (13).

During adrenal insufficiency, the signs are similar to those observed in hypophyseal asthenia but their duration is shorter and they precede death by only a few hours or a day at most. These signs appear from 1 to 3 days after destruction of the adrenals. Hypophyseal asthenia appears later and persists longer (1 to 8 or 10 days). It is more severe and typical than the adrenal asthenia (6).

Intestinal Absorption of Sugars During Hypophyseal or Adrenal Insufficiency in the Toad

Houssay, Foglia and Fustinoni (13) found that the absorption of glucose by the intestine of toads (*Bufo arenarum*) measured at 1, 2 and 3 hours, was normal during hypophyseal or adrenal asthenia even when the gastrointestinal tract showed a pronounced dilatation, hyperemia, and edema of the submucous layer.

Minebeck (14) has studied the absorption of different sugars by the intestine of *Rana esculenta* var. Hungarian. He observed a selective absorption of glucose and galactose which was two or three times faster than that of other sugars (fructose, mannose, sorbose and xylose). After destruction of the adrenals or extirpation of the hypophysis or of the anterior lobe[2] he found a disappearance of the selective absorption of glucose and galactose, the rate of absorption becoming equal to that of other sugars which were reabsorbed normally.

Minebeck's observations led us to perform new experiments on *Bufo arenarum* using different sugars after 8 hours of absorption.

[1] The principal lobe or pars glandularis or pars distalis of batrachians is structurally and functionally equivalent to the anterior lobe of mammals. But in the toad it is situated behind the neuro-intermediary lobe which is equivalent to the posterior lobe of mammals.

[2] The author calls this lobe anterior because it corresponds physiologically to the anterior lobe of mammals although it is situated behind the neuro-intermediary lobe in batrachians, corresponding to the anterior lobe of mammals.

Technic. Male toads from San Martin (Province of Buenos Aires) were used within 7 days of being captured. Females were discarded to avoid sex influences. All experiments were carried out in November (equivalent to May in the Northern Hemispheres). The toads were kept in concrete basins rinsed constantly by a feeble current of water. The temperature was between 18° and 20° C.

Under light ether anesthesia, a left paramedian laparotomy was performed. The small intestine was emptied of its contents by gentle digital expression. Two liga-

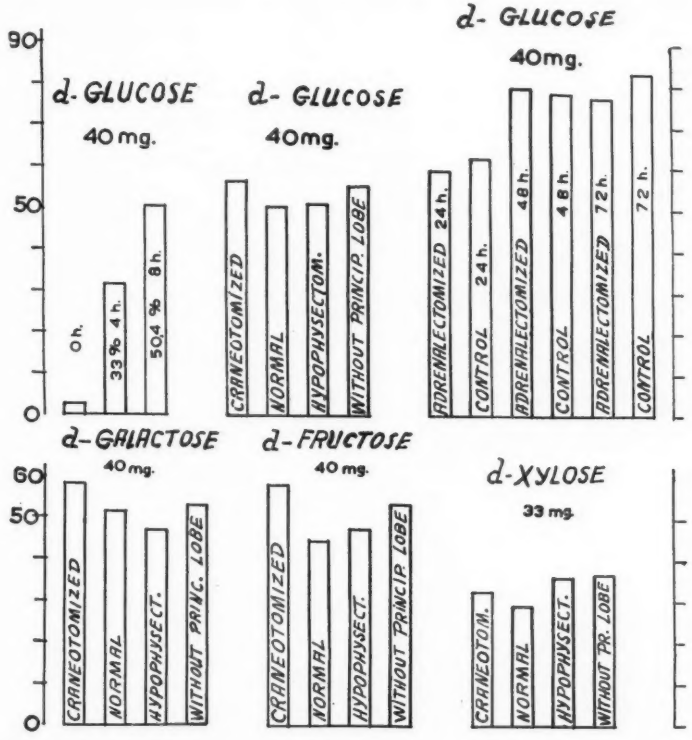

Fig. 1. INTESTINAL ABSORPTION OF VARIOUS SUGARS IN THE TOAD *Bufo arenarum*. Each column indicates the amount of sugar in percentage absorbed in 8 hours (except in the first 2 columns where the time is 0 and 4 hours). Hypophysectomies and removal of principal lobe performed from 13 to 20 days previously. The animals were asthenic.

tures were then put in place, one near the pylorus and the other near the large intestine.

By means of a long and carefully calibrated syringe with a fine needle, 1 cc. was injected through the pylorus into the intestinal lumen. Afterwards, a new ligature was placed on the pylorus without detectable loss of liquid. The following sugars were used: d-glucose and d-fructose of the British Drug Houses, galactose of Schuchardt, and d-xylose purified by Professor V. Deulofeu. Forty mg. were injected of each one of the first three sugars or 33 mg. of xylose dissolved in 1 cc. of distilled water.

Eight hours later the spinal cord was pithed and the intestine with the two ligatures was then removed. The intestine was opened and carefully rinsed with 5 cc. of water 3 times. The volume was made up to 25 cc. The amount of sugar was deter-

TABLE 1. TOADS, NOVEMBER 1. IMMEDIATE RECOVERY OF GLUCOSE INJECTED INTO THE SMALL INTESTINE

Controls			Glucose injected				
Weight of toad	Distilled water in intestine	Reducing power in mg. glucose	Weight of toad	Injected into intestine	Recovered	Absorbed	
gm.	cc.		gm.	mg.	mg.	mg.	%
120	1	1.3	120	40	38	2	5
118	1	0	125	40	38	2	5
125	1	0	122	40	39.1	0.9	2.5
117	1	0.5	118	40	38.2	1.8	4.5
124	1	2.3	110	40	39.7	0.3	0.8
115	1	0.5	115	40	40	0	0
113	1	0	115	40	40	0	0
		0.64					2.5±0.6

TABLE 2. TOADS, NOVEMBER 7. ABSORPTION OF GLUCOSE 4 HOURS AND 8 HOURS AFTER ITS INJECTION INTO THE SMALL INTESTINE

Controls			Glucose injected				
Weight of toad	Distilled water in intestine	Reducing power in mg. glucose	Weight of toad	Injected into intestine	Recovered	Absorbed	
gm.	cc.		gm.	mg.	mg.	mg.	%
			Four Hours After Injection				
124	1	2.1	120	40	30	10	25
110	1	0	140	40	27	13	32
104	1	0.5	115	40	25	15	37
121	1	0.5	122	40	28	12	30
116	1	0	120	40	25	15	37
			120	40	25	15	37
		0.6					33±1.3
			Eight Hours After Injection				
125	1	0	130	40	14	26	65
108	1	0	120	40	15	25	62
112	1	0	118	40	24	16	40
114	1	0.8	108	40	19	21	52
			100	40	17	23	57
			107	40	31	19	47
		0.2					54±0.8

mined in duplicate by the method of Hagedorn and Jensen using 0.2 cc. of fluid. The calculation was the same for glucose and xylose, but for galactose it was necessary to multiply the result by 1.20 and for levulose by 1.03.

In each experiment a control was set up by preparing 1 cc. of the solution of the sugar injected made up to 25 cc. of which 0.2 cc. was used for analysis.

Also each experiment was controlled by injecting 1 cc. of distilled water instead

of the sugar solution into the intestine of toads. Eight hours later the intestine was rinsed 3 times with 5 cc. of water. The volume was made up to 25 cc. and 0.2 cc. of fluid was taken for quantitative determination. Its reducing power was always found to be negligible (table 1, 2).

Recovery of injected sugar. It can be seen in table 1 that when 40 mg. of glucose were placed in the small intestine which was then immediately rinsed and analyzed, an average of 97.5% of the injected glucose was recovered.

Time of absorption. On introducing 40 mg. of glucose into the small intestine, there was an average absorption of 33% of the sugar at the end of the 4th hour and of 50% after 8 hours (table 2, 4, 6). We therefore chose the 8-hour period as did Minebeck.

TABLE 3. TOADS, NOVEMBER 13. ABSORPTION OF GLUCOSE 8 HOURS AFTER INJECTING 40 MG. IN 1 CC. OF DISTILLED WATER INTO THE INTESTINE. TOADS OPERATED UPON 13 DAYS BEFORE

Weight of toad	Glucose recovered	Glucose absorbed		Weight of toad	Glucose recovered	Glucose absorbed	
gm.	mg.	mg.	%	gm.	mg.	mg.	%
		Normal				*Craneotomized*	
100	21	19	47.5	100	24	16	40
100	21	19	47.5	95	18	22	55
120	23	17	42.5	120	24	16	40
115	18	22	55	105	18	22	55
100	20	20	50	100	24	16	40
				102	22	18	45
			48±1.3				45.8±2
		Hypophysectomized				*Principal lobe only removed*	
110	16	24	60	105	23	17	42.5
125	15	25	62.5	125	20	20	50
100	24	16	40	110	16	24	60
112	25	15	37.5	135	19	21	52.5
100	28	12	30	110	18	22	55
100	40	0	100				
			55±7				52±1.9

Intestinal absorption of various sugars in the normal toad. We have found the intestinal absorption of glucose and galactose to be similar in *Bufo arenarum* to that found by Minebeck in *Rana esculenta*. The absorption of xylose was much less than that of the previous two sugars, but slightly higher in this species of toad than in this species of frog. On the other hand, the absorption of fructose in the frog was only half that of glucose and galactose while in the toad it was only slightly less than that of the other two sugars. In *Bufo arenarum* there is a selective intestinal absorption of glucose with a quotient of glucose to xylose of 2.2 for the frog, and 1.72 for the toad. The strong selective absorption of fructose is peculiar to our toad but is not so to *Rana esculenta* (table 4, 5; fig. 1).

Intestinal absorption of sugars in toads with hypophyseal insufficiency. During the course of a day, the intestinal absorption of glucose or other sugars was compared simultaneously in several groups of toads: normals, craneotomized, totally hypophysectomized, and deprived of the glandular lobe only (equivalent to the anterior lobe of mammals). An example of these comparative experiments can be seen in table 3. Table 4 gives a complete summary. Two experiments were performed on

toads with signs of post-hypophysectomy asthenia, the first on those operated upon 13 days previously and the second 20 days previously.

The intestinal absorption of sugars was on the average normal in asthenic toads which had had a total hypophysectomy or removal of the anterior lobe 13 and 20 days previously. The average intestinal absorption of 40 mg. of glucose in 8 hours was 50% for 16 controls and 51% in 17 hypophysectomized toads (table 5; fig. 1).

The absorption of galactose and fructose was the same in hypophysectomized toads as in the controls. That of xylose was slightly greater in toads with hypophyseal insufficiency but the difference was not significant (table 4, 5; fig. 1).

TABLE 4. INTESTINAL ABSORPTION OF VARIOUS SUGARS IN THE INTESTINE OF *Bufo arenarum*, SPRING, NOVEMBER 1939

Hours after injection	Normal		Craneotomized		Principal lobe removed		Hypophysectomized		Days after operation
	No. of toads	Absorption, % Mean and σm	No. of toads	Absorption, % Mean and σm	No. of toads	Absorption, % Mean and σm	No. of toads	Absorption, % Mean and σm	
				d *Glucose*, 40 mg.					
0	6	2.5±0.6							
4	6	33.0±1.3							
8	40	50.4±0.8							
8	5	46.3±4.7	5	49.8±5.7	5	47.8±3.9	5	38.5±2.4	13
8	5	48.5±1.3	6	45.8±2.0	5	52.0±1.9	6	55.0±7.0	13
8	5	55.4±2.0	5	78.6±6.0	6	66.0±4.2	6	58.0±5.9	20
				d *Xylose*, 33 mg.					
8	10	28.7±3.3	9	32.4±5.7	11	36.5±7.2	15	35.6±4.8	13
				d *Galactose*, 40 mg.					
8	6	51.5±2.4	5	58.6±3.6	8	53.4±2.0	6	47.3±2.0	13
				d *Fructose*, 40 mg.					
8	9	44.0±3.0	9	56.0±3.1	10	56.0±3.3	10	45.7±3.7	13

These results hold for the toad *Bufo arenarum* and we should be careful not to commit the error of rejecting the experiments of Minebeck on *Rana esculenta* for we have not experimented on this species.

The selective absorption of glucose and galactose compared to that of xylose, is not altered by total hypophysectomy or by insufficiency of the pars glandularis. The glucose-xylose quotient is a little lower in those with hypophyseal insufficiency. This is not due to diminution of absorption of glucose but to an increase in the absorption of xylose.

The high absorption of fructose, peculiar to the toad, is also observed in the hypophysectomized as well as in the control animals (table 4, 5).

Absorption of glucose from the intestine of toads with adrenal insufficiency. After destruction of the adrenals of the toad (*Bufo arenarum*) by diathermy coagulation, most of the animals die between one and three days after the operation in spring (6). Controls subjected to a similar longitudinal cauterization of the kidneys leaving the adrenals intact, survive with no untoward affect. We have studied the absorption of

40 mg. of glucose by the intestine in 8 hours in toads operated upon 24, 48 and 72 hours previously. Most of these animals presented symptoms of asthenia. In each experiment, toads with the adrenals destroyed were compared with controls operated upon at the same time.

TABLE 5. AVERAGE SUGAR ABSORPTION BY THE SMALL INTESTINE OF THE TOAD *Bufo arenarum*, EXPRESSED IN PERCENTAGE IN 8 HOURS

	Glucose	Galactose	Fructose	Mannose	Sorbose	Xylose	$\frac{\text{Glucose}}{\text{Xylose}}$
Rana esculenta in Winter (Minebeck)							
Normal	55.4	52.6	20.5	20.7	10.0	24.5	2.20
Hypophysectomized	20.7	15.9	25.9	22.3	16.7	25.7	0.80
Rana esculenta in Spring (Minebeck)							
Normal	43.2	44.8	13.0	—	—	16.5	2.60
Adrenalectomized	15.7	12.7	13.4	—	—	12.2	1.28
Bufo arenarum (this paper)							
Normal	50.4	51.5	44	—	—	28.7	1.72
Hypophysectomized	51.2	47.3	45.7	—	—	35.6	1.43
Without principal lobe	55.9	53.4	56	—	—	36.5	1.53
Craneotomized	57.3	58.6	56	—	—	32.4	1.76
Adrenalectomized							
24 hr.	58.4						
48 hr.	79.4						
72 hr.	77.8						
Controls							
24 hr.	62.2						
48 hr.	78.2						
72 hr.	83.3						

The results presented in table 4 and 5 show that glucose is absorbed from the gut at the same rate in the toads in which the adrenals were destroyed as in the operated controls.

DISCUSSION

The experiments which are described confirm and extend the results of Houssay, Foglia and Fustinoni (13) in which the absorption of glucose was found to be normal in toads suffering from adrenal or pituitary insufficiency and where other signs such as asthenia and gastrointestinal congestion and edema were present. In these new experiments, the absorption of galactose, fructose and xylose was found to be normal (table 5). The discrepancy with the experiments of Minebeck may be due to the fact that a different batrachian (*Rana esculenta*) was used in his experiments.

We do not intend to challenge the theories of Verzar on intestinal absorption but it appears that our results do not fit easily into his schema.

In the experiments of Westenbrink and Gratama (15), Weel (16) and Minebeck (14), it was found that the frog's intestine absorbed glucose and galactose selectively and that other sugars (fructose, mannose, sorbose, xylose and arabinose) were absorbed much more slowly. The intestine of *Bufo arenarum* also absorbs glucose and galactose selectively, but fructose is absorbed nearly at the same rate.

SUMMARY

In the normal toad, *Bufo arenarum* Hensel, glucose and galactose are absorbed from the gut 1.7 times faster than xylose in 8 hours. Fructose is absorbed only slightly more slowly than glucose and galactose.

The absorption of these sugars was not found to be modified by insufficiency of the pituitary (total extirpation or extirpation of the principal lobe only, 13 to 20 days previously) or of the adrenals (destruction 1 to 3 days before) even when other symptoms such as asthenia and gastrointestinal dilatation were present.

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INHIBITION OF THE PSEUDOPREGNANCY REACTION IN RABBITS WITH ESTROGENIC AND ANDROGENIC HORMONES

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PALESTINE

IN MAN and most rodents ovulation takes place spontaneously. In the rabbit it normally occurs only after coitus. Copulation stimulates the anterior pituitary lobe to produce the luteinizing hormone, prolan B, which in turn stimulates follicle rupture and corpus luteum formation. Progesterone, which is produced in the corpus luteum, transforms the proliferative uterine mucosa into progestational (decidual) mucosa. In rabbits hypophysectomized within one hour after copulation, follicle rupture had not taken place. This indicates the role of the anterior pituitary gland in ovulation. When the pituitary peduncle is previously cut through, ovulation fails to follow copulation. It thus seems apparent that in sexually mature rabbits, the gonadotropic hormone can easily induce follicle rupture and secondarily corpus luteum formation through which the mucous membrane of the uterus is transformed into its progestational stage (pseudopregnancy) (1). That this entire reaction can be inhibited by means of estrogens has been shown by many authors for both animals and human beings (2). Sufficiently large quantities of estrogenic hormone can suppress the incretion of gonadotropic hormone such that premenstrual transformation of the uterine mucosa becomes impossible. If the pseudopregnancy reaction is of pituitary origin, then it should be possible to inhibit this reaction by estrogens. To confirm this, the following experiments were performed: 58 sexually mature rabbits were isolated for 4 weeks before use. A day before the beginning of the experiments laparotomies were made and the ovaries inspected. Only animals, with large mature follicles were used. A piece of one uterine cornu one centimeter long was excised and examined histologically in order to study its functional condition. Only those animals whose uterine mucosa was in the proliferative and not progestational phase were used. This indicates that no functioning corpus luteum existed.

To induce the pseudopregnancy reaction the electrical stimulation of the central nervous system, as described by Marshall and Verney (3), was used. One electrode was introduced into the rectum; the other, a long needle, was placed subcutaneously on the spinal process of the lumbar vertebrae. An alternating current of 30 volts was used. The stimulation for 3 seconds was carried out twice with an interval of 7 seconds. This was repeated 1 hour later. Six days after this stimulation the rabbits were killed, the ovaries examined macroscopically and the uterine mucosa microscopically.

In 5 of 8 rabbits (62.5%) the electrical stimulation caused secretion of gonadotropic hormone with concomitant formation of corpora lutea in the ovaries and progestational transformation in the uterine mucosa. These results correspond to those reported by Marshall and Verney, who obtained positive results in 66% of their experiments.

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In a second series the rabbits were treated for 5 days with estrogens in order to produce an inhibitory effect on the hypophysis and on the sixth day the electrical stimulation was performed. We employed estradiol benzoate.[1] The total dose given in 5 days varied between 1 and 15 mg. (table 1). 6 days after stimulation the animals were killed. The ovaries were found not enlarged, ovulation had not occurred and the mature follicles were rather smaller. In no case was there corpus luteum formation; the uterine cornu showed no peculiarities, the histological examination showed, in all cases, a proliferative phase. In no case did the progestational transformation, i.e., the decidual reaction take place.

TABLE 1. EFFECT OF ESTRADIOL BENZOATE ON THE PSEUDOPREGNANCY REACTION AFTER ELECTRICAL STIMULATION

Rabbit	Estradiol benzoate. Total dose		Electrical stimulation	Ovaries	Uterine mucosa	Pseudo-pregnancy reaction
	1 day	5 days				
	mg.	mg.				
1	0.5		8 hr.	large follicles	not progestational	negative
2	1.0		8 hr.	large follicles	not progestational	negative
3		1	6th day	large follicles	not progestational	negative
4		2	6th day	large follicles	not progestational	negative
5		3	6th day	no large follicles	not progestational	negative
6		4	6th day	no large follicles	not progestational	negative
7		5	6th day	no large follicles	not progestational	negative
8		10	6th day	no large follicles	not progestational	negative

From these experiments it is apparent that the administration of as little as 1 mg. of estradiol benzoate for 5 days inhibited pituitary function in the rabbit so that the electrical stimulation on the 6th day was unable to induce the secretion of gonadotropic hormone. In 2 other animals we tried to inhibit the hypophysis by a shorter treatment. The animals received single doses of 0.5, and 1 mg. of estradiol benzoate, and 8 hours later, on the same day, the electrical stimulation was carried out. This single dose of 0.5 to 1 mg. estradiol benzoate was also sufficient to inhibit completely the pseudopregnancy reaction; it is possible that the minimal dose is lower than 0.5 mg. although we were unable to determine this exactly.

Androgens. The androgenic hormone has an influence on the anterior pituitary lobe similar to that of the estrogenic hormone, e.g., small doses stimulate and large ones inhibit the gonadotropic secretion. In rats, anterior lobe could be stimulated by small doses, but not in the rabbit. Androgenic hormones as well as estrogenic hormones can delay menstruation and also inhibit lactation.

The experiments on inhibition of pseudopregnancy were made in the manner described below. First, 7 sexually mature rabbits were given from 1 to 20 mg. of testosterone propionate without subsequent electrical stimulation, in order to see whether testosterone propionate alone has a luteinizing effect on the rabbit ovaries. The results of these experiments were negative. No changes were found in the ovaries, and in no case was there a progestational transformation of the uterine mucous membrane.

In the next series, table 2, the rabbits were given testosterone propionate subcutaneously for 5 days in a total dose of 0.5 to 100 mg. On the sixth day the electrical stimulation was performed.

The results in table 2 indicate pseudopregnancy can not be prevented by 5 mg. of testosterone propionate (*rabbit 12*). With 10 mg. one positive reaction (*rabbit 13*)

[1] The estradiol benzoate and testosterone propionate were supplied by Roche-Organon, Inc.

and 2 negative ones (*rabbits 14* and *15*) were obtained. Ten mg. seems to represent the minimal dose, since with higher doses (20 to 100 mg.) the reaction was negative. It is thus possible to inhibit the pseudopregnancy reaction by estrogenic as well as androgenic hormone. However, the effective dose differs; it amounts to 0.5 mg. of estradiol benzoate, and 10 mg. of testosterone propionate.

TABLE 2. EFFECT OF TESTOSTERONE PROPIONATE ON PSEUDOPREGNANCY REACTION AFTER ELECTRICAL STIMULATION

Rabbit	Testosterone propionate. Total dose in 5 days	Electrical stimulation	Ovaries	Uterine mucosa	Pseudo-pregnancy reaction
	mg.				
9	0.5	6th day	Corpus Luteum	progestational	positive
10	1	6th day	large follicles	not progestational	negative
11	2.5	6th day	large follicles	not progestational	negative
12	5	6th day	Corpus Luteum	progestive	positive
13	10	6th day	Corpus Luteum	progestive	positive
14	10	6th day	no large follicles	not progestational	negative
15	10	6th day	no large follicles	not progestational	negative
16	20	6th day	no large follicles	not progestational	negative
17	20	6th day	no large follicles	not progestational	negative
18	100	6th day	no large follicles	not progestational	negative

Our experiments were performed between January and June. After this date the animals did not react, in that it was impossible to induce the pseudopregnancy by stimulating the hypophysis electrically, under standard experimental conditions. It is interesting that Marshall and Verney in England have also only been able to perform the experiments from January to June. The reason, consequently, has a decisive influence on the course of this reaction. Rabbits in Palestine are fecund during the whole summer, i.e., till November and also in winter in proper breeding conditions. Coitus is capable of inducing follicle rupture and the formation of corpus luteum while it is impossible to obtain the same experimentally. There must, therefore, be another unknown factor lacking in electrical stimulation and present in coitus stimulation.

SUMMARY

Follicle rupture, which does not occur spontaneously in the rabbit, can be brought about through coitus or electrical stimulation. These stimulations cause secretion of gonadotropic hormone from the anterior pituitary lobe, thus bringing about secondarily ovulation, corpus luteum formation and progestational changes in the uterine mucosa (pseudopregnancy reaction). This reaction can be inhibited by estrogenic as well as androgenic hormone. The minimal doses were found to be 0.5 mg. estradiol benzoate and 10 mg. testosterone propionate.

The pseudopregnancy reaction can be induced electrically only during the months of January to June. The reaction as a result of coitus can, however, take place at any time during the year. From this, we may conclude that a factor, yet unknown exists, responsible for a positive result with coitus and a negative result with electrical stimulation.

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EFFECT OF PITUITARY PREPARATIONS ON GLYCOGEN STORES OF FASTED MICE

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IT IS WELL KNOWN that extracts of anterior pituitary tissue exert a marked effect on the glycogen stores of fasted animals (1, 2, 3). On the other hand, reference has frequently been made to a relation of the posterior pituitary lobe and carbohydrate metabolism (4–8). Since we have found mice of the pure strain (dba) an excellent test object for the so-called glycotrophic or glycostatic effect of pituitary extracts (9), this method has been used to make a quantitative study of these properties in both anterior and posterior pituitary preparations. Dry powder pituitary protein hydrochlorides that do not appear to lose potency on storage in vacuo at room temperature have been prepared.

MATERIALS AND METHODS

Most of the studies reported were carried out on 5-to 6-week-old female mice of a pure strain (dba) and weighing from 10 to 14 gm. Unless otherwise stated, the method adopted as a standard procedure for the determination of the glycotrophic activity of pituitary preparations is as follows. The mice are fasted for 24 hours and the injections made subcutaneously in 3 equal fractions at 1, 6 and 12 hours respectively after the beginning of the fast. The animals are anesthetized with amytal, the liver removed quickly and the liver and carcass frozen in carbon dioxide ice. Further procedures including the analytical methods have previously been described (9). The usual amount of extract injected at any one time was 0.1 to 0.2 cc. In our experience, female mice give a far more uniform response than males. The average response, however, appears to be of the same order in both sexes.

Blood sugar determinations were carried out using Somogyi's modification of the Shaffer-Hartman method (10). Blood samples were obtained by decapitating the animals.

In the rabbits, guinea pigs and rats, liver and muscle samples for glycogen determinations were secured under amytal anesthesia. The method outlined by Bachman and Toby (11) was used for rabbits, while the method of Collip, Thomson and Toby (12) was used for rats and guinea pigs.

The anterior pituitary extracts were prepared from freshly frozen and carefully dissected glands, while the posterior pituitary extracts were prepared from frozen glands that had been standing in a frozen condition for varying lengths of time. Saline extracts were prepared by thorough extraction with physiological saline solution, using equal amounts of saline and anterior pituitary tissue, while for the posterior pituitary extracts 10 volumes of saline were used to one of tissue.

Protein hydrochlorides were prepared from both the anterior and the posterior

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pituitary tissues by the picrate-acetone method of Dodds and Dickens for pancreas (13) and as used by Dodds, Noble and Smith for pituitary and other tissues (14). The yield of hydrochloride from anterior pituitary tissue varied in different batches between 9.4 and 19 mg. per gm. of fresh tissue. Ash content varied between 0.31 and 0.60%, and nitrogen between 14.0 and 14.5%. The yield of hydrochloride from posterior pituitary tissue in two batches was 17 and 23 mg. per gm. fresh tissue, with an ash content of 1.86% and a nitrogen content of 13.3 and 13.6%. These products, light brown powders freely soluble in water, apparently retained their physiological prop-

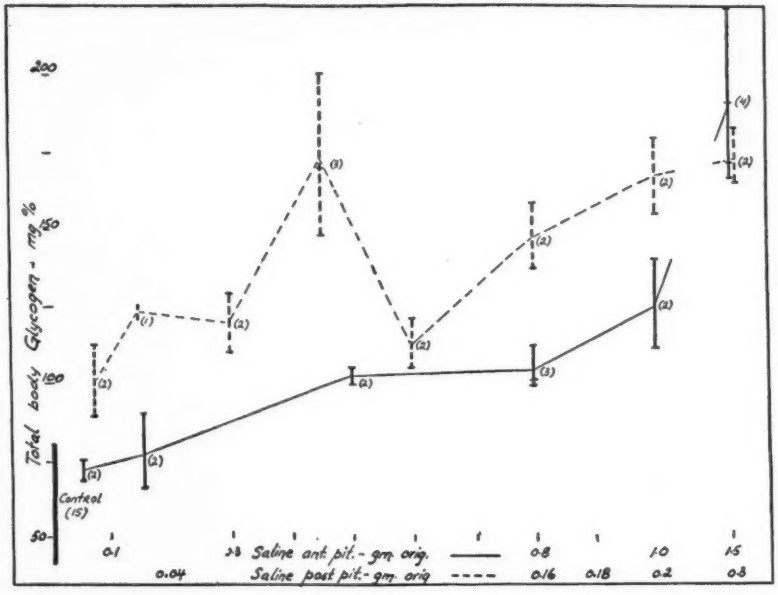

Fig. 1. THE EFFECT OF SALINE EXTRACTS OF ANTERIOR AND POSTERIOR PITUITARY TISSUE ON THE TOTAL BODY GLYCOGEN OF 24-HOUR FASTED MICE (dba) (the figures in parentheses show the number of analyses, vertical lines, the deviations).

erties at room temperature. The anterior and posterior pituitary protein hydrochlorides showed a positive biuret, xanthoproteic and Hopkins Cole reaction, and the presence of unoxidized sulphur. These tests were more marked with the posterior powder. Both products showed negative results with Million's and Molisch's tests.

Protein hydrochlorides were also prepared from fresh muscle tissue by the method outlined. These, together with physiological saline and saline muscle extracts, were used as the control preparations. The amount of muscle preparation used in the control animals was determined by its nitrogen content with reference to that of the pituitary preparations.

RESULTS

Figure 1 shows the effect of saline extracts of anterior as well as posterior pituitary tissue on the total body glycogen content of 24 hr. fasted mice, while figure 2 shows the effect of the protein hydrochlorides. The most striking feature of these results is the fact that the posterior pituitary preparations were far more potent than the cor-

responding anterior lobe extracts. With the saline extracts considerable variation in response was observed (fig. 1). Based on the weight of original tissue the posterior pituitary extract was at least 5 times as potent as the corresponding anterior lobe extract. On the other hand, with the powders (fig. 2), the posterior showed about 50 times the potency of the anterior lobe hydrochloride. Converting the amounts of the hydrochlorides used to their corresponding original weights, it appears that in the process of preparation the anterior had lost some of its potency but not to any appreciable extent, while the posterior had, if anything, increased in potency. This may have been due to the removal of some inhibitor. On the other hand, it is by no means certain that the saline extracts contained all of the activity present in the original tissue.

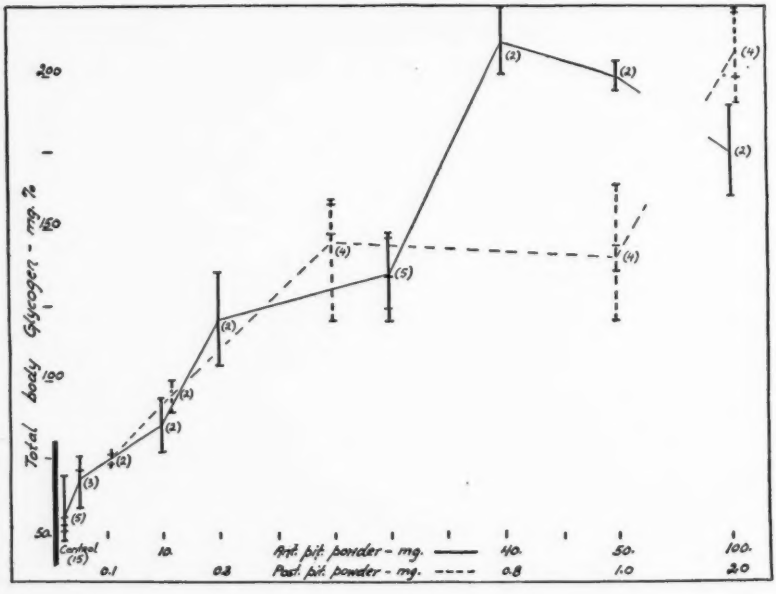

Fig. 2. The effect of anterior and posterior pituitary protein hydrochlorides on the total body glycogen of 24-hour fasted mice (dba) (the figures in parentheses show the number of analyses, vertical lines, the deviations).

We have omitted showing the glycogen levels of the liver and carcass separately since in our experience the effects observed with anterior as well as posterior pituitary extracts have been remarkably uniform. In the 24 hr. control animals the extremes of liver glycogen as the percentage of total body glycogen have been between 2.0 and 11.0%. On the other hand, in animals treated with pituitary preparations, and depending on the amount administered, the liver glycogen was increased up to 60% of the total body glycogen.

In table 1 the total body glycogen levels of fed as well as animals fasted for varying lengths of time are given. No appreciable loss of glycogen occurred during the first 5 hours of fasting. From 5 to 20 hours fasting 60% or more of the glycogen stores disappeared, evidently being used up for the energy needs. The glycogen levels remained constant from 20 to 48 hours of fasting.

In the experiments so far reported, the administration of pituitary preparations

was begun after the first hour of fasting. It may be that the effect was a restraint on carbohydrate oxidation per se. The 48 hr. fasted animals, however, that were treated with the pituitary preparation beginning at the 24 hr. fasting period, showed at least a 50% increase in total body glycogen. The extra glycogen must have been formed from some glycogen precursor. These results by no means exclude the possibility of a restraint on carbohydrate oxidation by pituitary extracts. They do, however, show that such preparations stimulate the production of glycogen from non-carbohydrate sources. The animals that were treated over the whole 48-hour fasting period showed a more marked response.

The results of previous investigators on the effect of posterior pituitary extracts on the glycogen levels indicated that they may be either diminished (4) or remain un-

TABLE 1. THE EFFECT OF FASTING AND OF POSTERIOR PITUITARY HYDROCHLORIDE ON THE GLYCOGEN STORES OF MICE (dba)

	No. of animals	Injection period	Dose	Total body glycogen	
				Range	Average
		hr.	*mg.*	*mg. %*	*mg. %*
Control, fed	6			203.0–322.0	262.0
Control, fasted 5 hr.	3			243.0–299.0	263.0
Control, fasted 14 hr.	3			68.0– 97.1	79.3
Control, fasted 20 hr.	3			52.1– 60.6	55.3
Control, fasted 24 hr.	15			40.3– 81.0	58.4
Control, fasted 48 hr.	4			59.7– 78.4	66.5
Fasted 48 hr.	8	24–48	2.0	103.0–142.0	128.0
Fasted 48 hr.	4	1–48	2.0–4.0	164.0–240.0	198.0

changed (5, 6). These analyses, however, were carried out within a few hours following the administration of the extracts. This suggested that in fasted animals posterior pituitary extracts initially produced a lowering of glycogen levels followed by an increase to supra-normal levels. The results in figure 3 support this view. The 24 hr. fasted animals did not appear to show any marked initial decrease in glycogen levels. They did, however, reach a uniform basal level. From 4 hours on, the values gradually increased until at the 16-hour period a definite effect was noticeable. At the 24-hour period (animals that were injected at the beginning of fast) the values are comparable to those given in figure 2. The effects observed will, therefore, chiefly depend on the time that has elapsed since the last injection. In the fed animals, a marked initial drop in the total body glycogen was observed. At the 1-hour period no liver glycogen was detectable with the methods used. The carcass glycogen levels also showed a marked decrease. These values apparently had returned to normal in 6 to 9 hours.

It is generally assumed that the administration of posterior pituitary preparations causes glycogenolysis which results in an immediate rise in blood sugar (14). In fed mice we observed a significant rise of blood sugar following the injection of the posterior pituitary hydrochloride (table 2). In the fasted group, only a slight immediate hyperglycemic response was noted, which may be associated with the insignificant drop in total body glycogen.

The effect of purified posterior pituitary preparations[2] on the total body glycogen levels of mice, together with the international standard posterior pituitary powder, are shown in table 3. The pressor preparation as well as the posterior pituitary stand-

[2] Dr. R. L. Stehle of the Department of Pharmacology, McGill University, kindly supplied us with some of his purified posterior pituitary preparations.

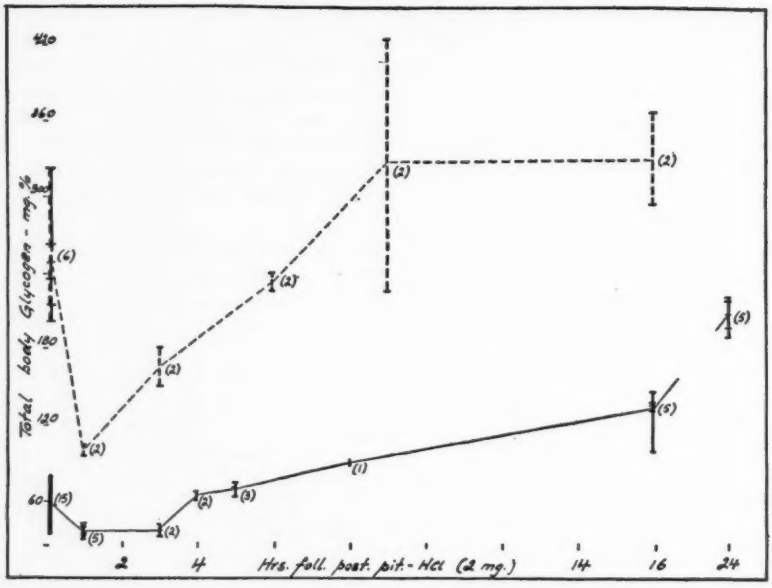

Fig. 3. THE TOTAL BODY GLYCOGEN CONTENT OF MICE AT VARIOUS PERIODS FOLLOWING THE ADMINISTRATION OF POSTERIOR PITUITARY HYDROCHLORIDE (solid line represents the animals fasted for 24 hours at the time of analyses; dotted line, fed animals).

ard showed a marked effect on the glycogen stores. No such effect was observed with the oxytocin.[3]

These results suggest that the glycotrophic property of posterior pituitary preparations may either be closely associated or identical with the pressor hormone. Assays of various pituitary preparations, however, do not suggest a close relationship between the glycotrophic and antidiuretic effects.

TABLE 2. THE EFFECT OF POSTERIOR PITUITARY PROTEIN HYDROCHLORIDE ON THE BLOOD SUGAR OF MICE (dba)

	Hours after extract	No. of animals	Blood sugar			
			Fed		Fasted 24 hr.	
			Range	Average	Range	Average
			mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.
Control	—	4	132–153	142	96–128	109
2.0 mg. subcut.	½	4	174–211	191	115–157	124
	1	4	186–211	196	107–141	122
	2	4	153–162	157	92–124	103
	3	4	132–174	152	96–137	111
	6	4	132–145	140	96–120	103
	8	3	162–190	172	100–111	104
	24	3	128–149	132	90–124	107

[3] The assay for the purified preparations as reported by Dr. Stehle are, 1 mg. pressor contains 200 pressor units and 10 oxytocic units, while 1 mg. oxytocin contains 250 U of oxytocin and 4 U of pressor substance.

TABLE 3. THE EFFECT OF VARIOUS POSTERIOR PREPARATIONS ON THE GLYCOGEN STORES OF 24-HOUR FASTED MICE

	Dose	No. of animals	Total body glycogen	
			Range	Average
	mg.		mg. %	mg. %
Dr. Stehle's oxytocin	0.015	2	44.8– 53.2	49.0
	0.03	2	32.2– 49.8	41.0
	0.06	2	60.1– 71.2	65.6
	0.12	2	44.8– 60.9	52.8
	0.30	3	40.7– 56.5	50.9
Dr. Stehle's pressor	0.015	2	58.4– 63.5	60.9
	0.03	2	116.0–172.0	144.0
	0.06	4	88.1–213.0	143.0
	0.12	2	204.0–236.0	220.0
	0.30	3	96.7–166.0	127.0
International standard	0.50	3	63.4– 75.6	68.0
	1.50	3	164.0–193.0	182.0
	3.00	3	94.1–206.0	151.0

Finally, it may be interesting to see whether the posterior pituitary preparations do show a glycotrophic effect in fasted animals other than mice (dba). Such experiments with rats, guinea pigs and rabbits are shown in table 4. The effects observed are not as marked as those shown in mice.

TABLE 4. THE EFFECT OF POSTERIOR PITUITARY PROTEIN HYDROCHLORIDE ON THE GLYCOGEN LEVELS OF RATS, GUINEA PIGS AND RABBITS

	Dose	No. of animals	Liver glycogen		Muscle glycogen	
			Range	Av.	Range	Av.
	mg.		%	%	%	%
Rats, fasted 24 hr.	—	5	0.18–0.89	0.48	0.412–0.472	0.454
	6–10	15	1.05–2.24	1.57	0.505–0.681	0.602
Guinea pigs, fasted 24 hr.	–	3	0.03–0.47	0.21	0.510–0.536	0.521
	8–12	9	0.23–2.66	1.50	0.575–0.752	0.648
Rabbits, fasted 28 hr.	—	1		0.55		0.336
	12–40	2	1.46–1.66	1.56	0.435–0.496	0.465

DISCUSSION

It may be appropriate to refer briefly to some of the other physiological properties of these pituitary protein hydrochlorides that have recently been reported (15, 16, 17). The anterior powder showed no evidence of appreciable amounts of thyrotrophic, gonadotrophic, and growth hormone. Some corticotrophic activity was demonstrated. The preparation showed a positive diabetogenic effect and it was antagonistic to insulin hypoglycemia. Marked prolactin activity was observed; in such species as the dog, rabbit and cat full mammary proliferation and lactation was observed in some instances. Most of the antidiuretic activity of fresh posterior pituitary tissue was present in the hydrochloride. In fairly large doses, this preparation showed, in rabbits, an antagonism to insulin hypoglycemia and adrenalin hyperglycemia.

It is well established that anterior pituitary extracts raise the glycogen stores of fasted animals. The mechanism of this action is as yet uncertain, and since the results reported do not give additional evidence they need no further discussion. We may point out that the anterior pituitary hydrochloride has been shown to be glycotrophic in fasted rats and rabbits as well as in mice.

In fasted mice (dba) posterior pituitary preparations showed a far greater glycotrophic activity than anterior lobe extract. This may suggest that the glycotrophic substance in anterior and posterior lobe tissue is the same, and further that the substance may primarily be of posterior lobe origin. No evidence for or against such a view is available. It is by no means certain that the initial drop in glycogen stores following the administration of posterior pituitary preparations was caused by the glycotrophic substance. The evidence rather favors the view that this may be an effect of the pressor substance (7). No such initial drop in glycogen stores has been observed with anterior lobe preparations. In other species such as the rat, guinea pig and rabbit, variations in the activity of anterior and posterior lobe extracts are not as marked as those observed in the mice.

It has been shown that in fasted mice the glycotrophic substance causes formation of new glycogen from non-carbohydrate sources. In treated animals 30 to 60% of the body glycogen stores were present in the liver compared to 2 to 11% in control animals, showing that the effect was primarily on the liver. With regard to glycogen stores and distribution the fasted treated mice appeared more or less like normal fed animals; in the latter, liver glycogen represented between 14 and 32% of the total body stores. In fed mice supra-normal levels of glycogen stores have not been observed.

In fasted rats, guinea pigs and rabbits, the effect of posterior pituitary preparations on the glycogen stores was not as marked as in mice. The dose and time of maximum response have not as yet been thoroughly investigated. It is quite possible that other factors as yet obscure may be responsible for this variation in response.

SUMMARY

It has been shown that in fasted mice pretreatment with either anterior or posterior pituitary preparations resulted in a marked increase in total body glycogen stores. The effect appeared to be mainly on the liver and due at least in part to new glycogen formation from non-carbohydrate sources. Posterior lobe preparations showed a more marked effect than the corresponding anterior lobe extract. The substance responsible for this effect was present in a purified pressor preparation and in the international standard powder. In the doses used, the purified oxytocin preparation was ineffective.

Posterior pituitary preparations showed a glycotrophic effect in fasted rats, guinea pigs and rabbits. This effect was not as marked as that shown by mice.

Anterior and posterior pituitary protein hydrochlorides have been prepared that retain their physiological properties at room temperature.

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ANTERIOR PITUITARY HORMONES WHICH FAVOR THE PRODUCTION OF TRAUMATIC UTERINE PLACENTOMATA[1]

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LOEB (1), who discovered the capacity of the endometrium to produce structures resembling the maternal placenta when traumatized or brought in contact with foreign bodies, showed that the production of these structures was so limited with reference to the phases of the sex cycle as to lead him to the conviction of the essentiality of corpus luteum function for this phenomenon. Long and Evans (2) made the first painstaking attempt to transfer Loeb's experience to the rat, a form characterized by so short an estrous rhythm as to forbid any appreciable length of time for function of the cyclic corpora lutea. Their experience harmonized with the Loeb conception, for they were unable to produce these growths at any time in the normal short cycle (4 to 6 days) of these rodents. On the other hand, either physiological or artificial means of prolonging the life of the corpora lutea (lactation or induction of pseudopregnancy) made it possible to establish typical uterine structures of this sort. Inasmuch as the last mentioned authors had seen the artificial production of long diestrus and abundant corpora lutea (mulberry ovaries) by parenteral treatment of normal animals with anterior pituitary extracts, the way was clear for Teel (3) to show that placentomata were readily produced during such treatment. He showed further that these procedures were futile after ovariectomy, proof that the pituitary hormones were not acting directly on the uterus but only through their influence on the ovary.

It remained for Weichert (4) to show that these endometrial responses were in fact due to the action of the two ovarian hormones cooperating with a mechanical stimulus to the endometrium. When he selected the proper dosage with these two hormones and gave them in the proper sequence, he could produce these growths in spayed females. Weichert employed corpus luteum extracts. Both Astwood (5) and Rothchild, Meyer and Spielman (6) were able to employ pure progesterone as well as estradiol in such experiments. Although the last mentioned authors found some definite advantage attached to the administration of small doses of the estradiol in addition to progesterone, Astwood was unable to detect this advantage. Both authors agree that high estrogen dosage inhibits placentoma formation.

It would hardly need to be stated that pituitary influences on the gonads are still imperfectly understood. The types and characteristics of gonadotrophic hormones are unsettled problems. Nor are we adequately informed as to possible gonadal effects of other hypophyseal hormones. Progress in purification of the lactogenic hormone in

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this laboratory afforded an opportunity of testing its potentialities in this respect. Such a scrutiny was especially called for, inasmuch as Dresel (7), Lahr and Riddle (8), and Nathanson, Fevold and Jennison (9) had all shown a suppression of the estrous cycle for at least 10 or 12 days in mice and rats on continuous daily dosage with the lactogenic hormone, a phenomenon we have confirmed. The suggestion has been made that we have in this result an explanation of the lactation anestrum of mammals. All of the above mentioned observers and we, ourselves, have detected large and apparently healthy corpora lutea in the ovaries of the animals subjected to lactogenic hormone, an added feature of the phenomenon being the production of the typical mucin-producing vaginal epithelium of pregnancy.

METHODS

Both normal and hypophysectomized rats were used for these tests. The normal animals were 5 to 7 months old, weighing 250 to 270 gm. Animals were hypophysectomized at 65 to 75 days of age when weighing from 140 to 180 gm. At time of autopsy the sella turcica of all operated animals was searched for remaining fragments of the pituitary. Only animals thus proven to be completely hypophysectomized were included in the tables. As these animals were mature at time of operation, corpora lutea were present. Such corpora lutea persist in the hypophysectomized rat for long periods unless substances are injected which cause their dissolution. Injections were begun in hypophysectomized rats on the day of operation unless otherwise specified. It was deemed essential to submit the animal to the hormone being investigated for 5 days preceding insertion of threads into the endometrium and for 5 days thereafter, autopsy being on the eleventh day after the beginning of injection.[2] In all cases two loops of silk thread were inserted through the uterine wall in such a way as to have each thread actually traverse the lumen for a short distance. In this way, enough of the endometrial surface was contacted to make these procedures an adequate test of the capacity of the tissue to respond to the foreign agent. In dealing with animals possessing a pituitary, only animals with a normal vaginal rhythm were used. Vaginal cycles were followed during the injection period. In some instances the vagina was taken for section at autopsy—a desirable check upon the daily study of vaginal smears. At autopsy, both uterine horns were observed under the binocular microscope and careful inspection of the ovary made after opening the ovarian bursa. In all cases microscopic examinations were made of these portions of the reproductive system, particular attention being paid to the endometrium at the site of threads. In the study of the ovary attention was directed to the number and condition of the follicles and corpora lutea, and in hypophysectomized rats to the interstitial tissue as well.

Under the experimental conditions defined above, 85% of the normal adult rats (37 animals) formed placentomata about threads when injected daily intraperitoneally with alkaline extracts[3] of beef anterior pituitary in doses containing approximately 17 mg. dry substance per cc.

The insertion of threads into the uteri of untreated rats, 6 of which were normal and 13 hypophysectomized, did not result in visible uterine swellings or in any trace of placentomatous tissue about the threads.

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[2] Hypophysectomized rats accordingly received threads day 4 and were autopsied day 10 postoperative.

[3] Such extracts are rich in growth hormone, but also contain considerable amounts of ICSH, lactogenic, adrenocorticotrophic and thyrotrophic hormones. It will be shown later that lactogenic hormone is very effective in stimulation of placentomata. Growth hormone freed of lactogenic hormone by cysteine treatment was not effective in stimulating placentomata in hypophysectomized rats when given in 0.1 mg. daily doses for 10 days (6 rats). The cysteine treated growth hormone was prepared by H. Fraenkel-Conrat.

TABLE 1. INFLUENCE ON PLACENTOMA FORMATION OF SHEEP PITUITARY GONADOTROPHIC PREPARATIONS HIGH IN LUTEINIZING EFFECTS

Type of rat	Preparation[1]	Total dose[2] in 10 days, subc.	Placentomata No. of rats		Ovaries	
			Positive	Negative	Av. wt.	Histology[3]
		mg.			mg.	
Normal	A	16.70	2	1	134	Many s, m CL (few degenerating) almost no follicular growth
		1.67	0	3	114	Several s, m CL, some degenerating; few m-lF
	B	8.30	0	3	101	Many s, m, l CL, almost none degenerating; few m, m-lF
		0.83	0	3	80	Few s, m, l CL, some degenerating; few m, m-lF
	C	8.30	0	3	86	CL, some degenerating; CL cysts (thecal); few lF
		0.83	0	3	68	CL, some degenerating; few mF
	Control	—	0	6	65	Several CL, some degenerating; developing F; IT normal
Hypophysectomized	A	16.70	0	3	118	Many healthy CL, all sizes; mF; IT repair
		1.67	0	3	39	Many s CL; few s, m F; partial repair of IT
	B	8.30	0	3	64	Many s, l CL; few lF; partial repair of IT
		0.83	0	2	49	Few CL; s, m F; IT deficient
	C	8.30	0	3	46	Few s, m CL, some degenerating; few s, m, l F; partial repair IT
		0.83	0	3	30	Several CL, several regressing; s, mF, usually in atresia; IT deficient
	Control	—	0	6	38	Several persistent CL, not degenerating; no development of F; IT deficient

[1] Three different preparations (A, B, C) from sheep pituitary were used; all were relatively crude preparations made by flavianic acid or ammonium sulfate precipitation. Preparation A and B produced CL in hypophysectomized immature rats in 72 hr. at 5 mg., preparation C at 2.5 mg. doses.

[2] Administered here, and in all subsequent tables unless otherwise specified, in 10 daily injections, 5 before insertion of threads in uterus and 5 after threads inserted.

[3] Abbreviations used are as follows: F=follicles or follicular; CL=corpora lutea; IT=interstitial tissue; s, m, l refer to the size, small, medium or large of ovarian structures.

Rat pituitary implants. Normal test animals (3 per group) received implants of rat pituitary for 3 days (instead of the usual 5 days) before threads were inserted; the daily implants were continued for 5 days, followed by autopsy on the sixth day. When implants of 2 rat pituitaries were given daily, 3 of the 4 rats implanted developed placentomata. When 4 and 8 implants, respectively, were given daily, no placentomata were produced. The average weights of ovaries in the 3 groups were 113, 123, and 139 mg. respectively. Large follicles as well as corpora lutea were found in the ovaries in all cases.

It is apparent that rat pituitary implants were not, in the amounts employed, an efficient stimulus to the formation of placentomata about threads inserted into

the endometrium. Inasmuch as these implants are well known to stimulate not only the formation of corpora lutea but also the growth of follicles with consequent production of estrus, the difficulty with which placentomata are produced under these conditions might be explained by the adverse effects of the estrogenic substance produced.

Pituitary gonadotrophic hormone high in luteinizing effects. Working on the assumption that rat pituitaries contain too high a proportion of FSH and therefore caused the production of excess estrogens, sheep gonadotrophic hormone which luteinized the

TABLE 2. INFLUENCE OF PITUITARY FOLLICLE STIMULATING HORMONE[1] ON PLACENTOMA FORMATION

Type of rat	Fraction injected	Total dose in 10 days, subc.		Placentomata No. of rats		Ovaries	
		mg.	U	Positive	Negative	Av. wt. mg.	Histology
Normal	A	10	10	0	3	86	Few CL; few s, m, lF
		50	50	2	1	147	Few CL, some very vascular; s, m, lF
	B	83	30	1	2	96	Few 1 CL (medium sized cells), m, lF
Hypophysectomized	A	10	10	0	3	49	Sev. CL (medium sized cells); s, mF; IT deficient
		50	50	0	3	117	M, 1 CL; lF and cysts; IT deficient

[1] The rat unit of FSH used was the amount giving significant augmentation (50–100%) when injected subcutaneously combined with chorionic gonadotrophin (given alone, ovarian wt., 35 mg.) autopsy at 72 hr. This dose is approximately the same as that necessary to cause resumption of follicular growth in hypophysectomized female rats (26–28 days at operation, 8–10 days postoperative). The preparations were made by flavianic acid, tannic acid and ammonium sulfate fractionation of sheep pituitary extracts. The unit varied from 1.0 to 2.5 mg. wet (SAS) cake. About 10-fold this dose (72-hour test) caused beginning repair of IT and CL formation. The preparations were free of lactogenic and thyrotropic hormones at 100-fold, the M.E.D. judged by injection into immature pigeons. They caused no gain in body weight and no repair of adrenals or thyroids of hypophysectomized rats when injected in a total dose of 175 M.E.D. over a period of 25 days.

follicles was tested for placentoma formation in both normal and hypophysectomized rats (table 1).

The results of the injection of such preparations into hypophysectomized animals were completely negative. Even in normal animals placentoma formation was rare following this treatment (2 of 18 rats). Study of the ovaries of the injected hypophysectomized rats showed that in addition to the corpora lutea present at time of hypophysectomy, other corpora lutea had been formed. The ovarian weights of the injected operated rats exceeded those of hypophysectomized controls (38 mg.) and were frequently greater than those of normal females (65 mg.), reaching a maximum of 156 mg. (the average for the group cited being 118 mg.).

The primary criterion for a luteinizing hormone was satisfied by these preparations, that is, they produced corpora lutea; yet in spite of this histogenetic effect, it would appear that the lutein tissue did not pour out any considerable amount of progesterone. It will be shown later that judging from placentoma formation, lactogenic hormone stimulates the secretion of progesterone-like substances from such artificially induced corpora lutea. The failure of these luteinizing preparations to stimulate sufficient function of such lutein tissue seems paradoxical. It must be borne in mind, however, that estrin secretion here, too, may have interfered with the decidual response.

TABLE 3. INFLUENCE OF HYPOPHYSEAL INTERSTITIAL CELL STIMULATING HORMONE[1] ON PLACENTOMA FORMATION

Type of rat	Fraction injected	Total dose in 10 days, IP		Placentomata No. of rats		Ovaries	
		mg.	U	Positive	Negative	Av. wt., mg.	Histology
Normal	A	24	10	0	3	68	Several CL, all sizes
		48	20	1	1	71	Several s, m CL (m sized cells); few s, m, lF
	B	83	3	3	0	89	Several CL (m cells); also CL cysts
		250	10	7	0	120	Same
	C	250	10	3	0	135	Several s, m CL; few s, m F
		250	10	3	0	117	Several l CL; some lutein cysts
	F	1	100	0	4	87	s, mF; CL in different functional states; IT normal
Hypophysectomized	A	24	10	0	6	47	Several CL, some normal and some degenerate; IT partially repaired
		48	20	0	2	27	CL partially degenerate; IT partially repaired; no F enlargement
	B	250	10	0	3	21	Remnants of degenerating CL; IT repaired; no F growth
	D	25	10	0	6	39	CL cells medium and irregular size; some CL show definite atrophy; IT repaired, but not hypertrophied
	E	0.125	10	0	4	30	l CL, cells uniform; some degenerating; IT repair
		0.250	20	0	3	40	l CL, cells uniform; few degenerating; partial IT repair
	F (threads)	1	100	0	4	41	No F dev.; healthy, persistent CL, also older resorbing CL; IT repaired
	F (no threads)	1	100	0	4	37	No F dev.; CL as in preceding; IT repaired

[1] The preparations *A*, *B* and *C* were made by flavianic acid and ammonium sulfate fractionation. They were kept as wet ammonium sulfate cakes without dialysis. Dose of 2.5 to 25 mg. wet cake was necessary to repair the IT of hypophysectomized rats (25–27 days at operation, 8–10 days postoperation) at onset of injection; autopsy 72 hr. after onset of the 3 daily IP injections. The only effect of these preparations on the gonads, even when given in 200 mg. doses over 25 days to hypophysectomized male and female rats was IT repair; in the male this was accompanied by some male accessory organ growth. Preparation *B* gave a crop gland reaction in 33-day-old pigeons when injected intrapectorally at 100 mg. doses; preparations *A* and *C* were still negative for lactogenic hormone at this level. The minimum dose for thyrotrophic hormone (pigeon) was above 100 mg. Preparation *E* and *F* were of higher potency than the preceding preparations. They were prepared by ammonium sulfate fractionation, were then freed of salt and converted into dried powders. Preparation *F* was prepared by Dr. C. H. Li, and proved to be homogeneous in the Tiselius apparatus (12).

Cornified vaginal smears were frequently present in both normal and hypophysectomized rats during the experimental period.

The complete absence of placentoma formation in hypophysectomized rats injected with these preparations is probably more significant than the very occasional

TABLE 4. STIMULATION OF PLACENTOMATA AROUND THREADS IN UTERI OF NORMAL AND HYPOPHYSECTOMIZED RATS ON ADMINISTRATION OF PREGNANT MARE SERUM GONADOTROPHIC HORMONE AND ON ADMINISTRATION OF CHORIONIC GONADOTROPHIN

Type of rat	Total dose in 10 days		Placentomata		Ovaries	
			No. of rats		Weight, mg.	Histology
	mg.	U	Positive	Negative		
	IP		*PMS Gonadotrophin*[1]			
Normal	50	10	0	3	90	Several l CL (m cells)
	500	100	0	3	640	Many l CL (l cells) and lutein cysts
	250	250	0	3	562	
	500	500	0	3	518	
	5000	1000	0	3	660	
Hypophysectomized	50	10	0	3	62	Few l CL (s-m cells); no F development; IT normal or hypertrophic
	500	100	0	3	502	Many l CL and cysts (follicular and lutein); IT hypertrophic
	250	250	0	3	197	
	500	500	0	3	366	
	5000	1000	0	4	385	
	subc.		*Chorionic Gonadotrophin*[2]			
Normal immature	0.05	10	0	6	53	4–6 CL; m, m-l F
	0.1	20	0	6	56	
	10.0	50	0	4	174	Many CL of different ages; few lF
	50.0	1000	0	3	161	
Normal adult	13.7	10	0	3	74	Sev. normal CL, few degenerating CL
	137.0	100	0	3	304	Increased number of l CL (l cells); CL cysts (thecal)
	1370.0	1000	1	2	205	
	50.0	1000	1	2	245	
	250.0	5000	0	3	253	
Hypophysectomized mature	13.7	10	0	3	47	Sev. l CL (s cells); no F devel.; IT repaired partially
	137.0	100	0	3	71	Sev. l CL (s cells); no F devel.; IT repaired
	50.0	1000	0	5	63	
	250.0	5000	0	3	53	

[1] The unit used for PMS gonadotrophin is the minimum amount stimulating follicular growth and development of estrous uterus in the normal immature rat (3 daily intraperitoneal injections, autopsy 96 hr.)

[2] The unit used for chorionic gonadotrophin is the total dose necessary to produce at least one CL in the ovary of one of three 24–26 day old rats when given in 3 subcutaneous injections followed by autopsy in 96 hours. The chorionic gonadotrophin injected was obtained from the I. G. Elberfeld, and from E. R. Squibb, both of which firms we desire to thank.

formation of placentomata in the normal animals which were injected with these fractions.[4] It must, of course, always be remembered that the injection of hormones into normal animals may induce the pituitary gland itself to contribute an unusual amount of one or more of its hormones; hypophysectomized animals are for this reason essential for the interpretation that any substance has exerted a direct effect on the ovary.

Follicle stimulating hormone (FSH). It could easily be presupposed that a purified FSH would not be active in these experiments, as estrin might be expected to interfere with the production of placentomata. FSH was, nevertheless, included in the tests. The FSH was prepared from sheep pituitaries by ammonium sulfate fractionation and by the use of ammonium sulfate with flavianic acid and tannic acid (10, 11). In table 2 it will be noted that 3 of the 9 normal animals developed placentomata, while none of the 6 hypophysectomized animals responded, although they received the same dose levels. The ovaries of the hypophysectomized rats responded readily by follicular growth and in some cases by luteinization; ovarian weights averaging 117 mg. were secured.

Interstitial cell stimulating hormone (ICSH). It has been possible to show that by appropriate chemical preparation of pituitary extracts, a gonadotrophic fraction can be separated which selectively stimulates or repairs the interstitial tissue of the ovary or testis of the hypophysectomized rat—the interstitial cell stimulating hormone (ICSH) (10, 11, 12). As table 3 shows, placentomata were frequently produced in traumatized uteri when 3 to 20 U of less purified ICSH were given to normal animals (17 of 21 rats) but not in the normal rats receiving the purest preparation of ICSH. It is highly interesting that, in spite of the presence of placentomata in such animals, the vaginal smears often indicated a normal estrous rhythm. Healthy corpora lutea of various sizes were present in the ovaries. It will be noted that the most potent and purest ICSH did not induce placentomata in normal animals even in much higher doses and that none of the preparations induced placentomatous growths about uterine threads in hypophysectomized rats. The more impure preparations of ICSH appeared to cause regression of the persistent corpora lutea of the hypophysectomized rats. It is noteworthy that these preparations, especially the purest ones, had an extremely low content of lactogenic hormone.

Pregnant mare serum gonadotrophic hormone. It is well known that the very potent gonadotrophic agent found in the blood stream of pregnant mares leads to the formation of many large ovarian follicles as well as lutein tissue and it might therefore be expected that placentomata would be difficult or impossible to produce when large doses of the pregnant mare serum hormone were employed. Table 4 shows this to be the case. In groups receiving higher doses, strikingly heavy ovaries were produced containing a large amount of lutein tissue, the individual cells of which resembled or exceeded in size those characteristic of pregnancy, yet placentomata were not produced. The results were the same in hypophysectomized rats.

Attention should be called to the fact that although placentomata were not formed when pregnant mare serum was administered, nevertheless a series of highly characteristic changes were found in the endometrium of the treated animals. These consisted of (*a*) a marked edema of the stroma separating the cells, (*b*) the production of folds and villous-like projections of the endometrium. Inasmuch as both these changes are encountered in the area adjacent to a true placentoma and occur in the area about

[4] Spontaneous formation of placentomata is occasionally noted, though no threads have been inserted, in normal adult females injected with standard alkaline extract of the pituitary or high doses of lactogenic hormone (1 mg. daily). Both types of treatment cause prolonged diestrus.

TABLE 5. STIMULATION OF PLACENTOMATA AROUND THREADS IN UTERI OF NORMAL AND HYPOPHYSECTOMIZED RATS ON ADMINISTRATION OF LACTOGENIC[1] AND ADRENOCORTICOTROPHIC HORMONES

Hormone	Type of rat	Preparation	Total dose in 10 days, subc.		Placentomata No. of rats		Ovaries	
			mg.	I.U.	Positive	Negative	Weight, mg.	Histology
Lactogenic	Normal	A	2	35	0	3	65	Few vascular CL; no F devel.
			10	165	3	0	61	Few l CL (m-l cells, abundant cytoplasm); few mF
			20	330	3	0	56	
		B	60	600	3	0	77	
	Hypophysectomized		5	125	2	1	22	Sev. l, large-celled CL in rats where placentomata present. In neg. cases CL absent in one, resorbing in the other; IT deficient
		C	10	250	4	0	27	Sev. l, large-celled CL in each ovary; IT deficient
		D	50	1250	4	0	33	Few l CL (cells large, abundant cytoplasm); no F dev.; IT deficient
Adrenocorticotrophic	Normal	E	2	R.U. 0.1	0	3	73	Few ml CL, vascular (cells less even in size and less tightly packed than in higher levels); few s and lF
			10	0.5	3	0	56	Few l healthy CL (cells large) few mF
			20	1.0	3	0	60	
		F	100	3.3	3	0	93	
	Hypophysectomized	G	50	2.6	1	1	33	Few l-celled CL in positive case; no F dev.; partially deficient IT
		H	110	4.2	4	0	28	Few m and l CL (few degenerating); no development; IT deficient

[1] The lactogenic hormone was prepared by W. R. Lyons by the method described in 1937 (13). The potency of the preparations (A to D) is expressed in terms of I.U. (although the international standard was not available when the earlier, less potent preparations were standardized, approximate values have been derived by translation from other standards). These preparations had a unit of adrenocorticotrophic hormone in 60 to 90 mg., except the preparation (C) which did not stimulate adrenal cortical growth in hypophysectomized female rats in a total dose of 50 mg. when given during 10 days, whereas 0.5 mg. total dose of impure adrenocorticotrophic hormone has been shown to accomplish this result. The lactogenic hormone is free of thyrotrophic effect in immature guinea pigs at 100 mg. doses (morphological and functional tests). Only slight growth was produced in hypophysectomized rats even at 50 to 200 mg. doses. The preparations were free of gonadotrophic effects at very high doses (see table 7). The adrenocorticotrophic hormone was prepared by H. D. Moon by the method described in 1937 (14). The unit of ACT was the amount of material which, administered to 21-day-old male rats in 3 subcutaneous injections (followed by autopsy at 72 hours), caused an average increase of 50% in the weight of the adrenals. These preparations contained 2.5 to 5.0 I.U. of lactogenic hormone per mg. There was no detectable contamination with growth hormone and no thyrotrophic hormone in total doses of 100 to 150 mg. given in 10 days to hypophysectomized rats. The preparations caused also no repair of the ovaries of hypophysectomized rats at these doses.

the inserted threads when too low a dose level is given of certain preparations (adrenocorticotrophic, lactogenic) which at higher levels produce placentomata, the assumption may be made that these are the preliminary stages in placentoma formation, though actual transformation of the stromal cells into decidual cells did not occur.

Human chorionic gonadotrophin. The principle found in the urine of pregnant women has been reported to facilitate placentoma formation in normal animals, but under the conditions of this study has been found to be a relatively poor stimulus to placentoma formation in both normal immature and normal adult rats though a considerable range of dosage was tested. As anticipated, the substance led to the formation of corpora lutea in the ovaries of immature test animals, and in normal adults caused an increase in the number of corpora lutea, also follicle growth and cyst formation with or without luteinization of the wall. Table 4 shows the infrequency of

TABLE 6. PLACENTOMA FORMATION AROUND THREADS IN UTERI OF ADRENALECTOMIZED AND OVARIECTOMIZED RATS ON ADMINISTRATION OF LACTOGENIC AND ADRENOCORTICOTROPHIC HORMONES

Type of rat	Total dose in 10 days, subc			Placentomata		Ovaries	
		Units of hormone[1]		No. of rats			
		Lactogenic	Adrenocorticotrophic	Positive	Negative	Weight	Histology
	mg. 60	I.U. 300	R.U. 1	3	0	mg. 58	Several l, healthy CL
Adrenalectomized[2]	50	500	5/6	2	1	44	Positive cases: several l, healthy CL. Negative case: corpora degenerating, no F development
Ovariectomized	50	500	5/6	0	3	—	

[1] For definitions see table 5.

[2] Injections were begun on the day rats were adrenalectomized or ovariectomized. Insertion of threads and autopsy were performed at the usual intervals.

placentoma formation in both normal adult and normal immature rats,[5] being produced in 2 only of 34 normal animals injected with total doses over 10 days varying from 10 to 5000 R.U. No placentomata were produced in the hypophysectomized animals receiving corresponding doses. An abundance of large corpora lutea, as well as hypertrophied interstitial cells were present in the ovaries of these animals (mature at time of operation). There was continuous desquamation of cornified cells from the vaginal walls, probably due to estrin production by the greatly hypertrophied interstitial cells. It is known that such animals breed. The contention of the presence of estrogenic substances in the preparation of chorionic gonadotrophin is hardly tenable since a purified product was employed.

Lactogenic hormone. Highly purified lactogenic hormone (13) has been employed and studies were made of its effects on the function of the normal reproductive tract, as well as on the production of foreign-body placentomata. The former observations will be reported in more detail later but in brief it can be said that the administration of certain levels of the hormone always led to the disappearance of normal cyclic changes in the vaginal smear, the latter showing the persistent presence of leucocytes, epithelial cells and mucus in the proportions seen in pregnancy; sections of the vaginal mucosa showed a striking histological correspondence with the conditions seen in

[5] In the normal animals, there were indications of some endometrial response, since villous formation similar to that observed on the injection of pregnant mare serum was observed.

normal pregnancy. Examination of the ovaries during this interval disclosed the persistence of corpora lutea already present at the time administration of the hormone was begun; the corpora were large and were composed of large cells. There was no evidence of the new formation of lutein tissue or of maturing of follicles. The ovaries of hypophysectomized rats injected with lactogenic hormone were characterized also by the presence of a few corpora lutea composed of large clearly outlined cells (fig. 1). No follicular growth occurred and the interstitial cells were atrophic. Table 5 shows

TABLE 7. TESTS SHOWING FREEDOM OF LACTOGENIC HORMONE FROM CONTAMINATION WITH GONADOTROPHIC HORMONES

Test animal	Length of injection	Total lactogenic hormone		Route of injection	Av. wt. of gonads
	days	*mg.*	I.U.		*mg.*
Normal immature female rats[1]	3	60	1500	Subc.	16
	3	60	1500 (+chorionic gonadotrophin)	Subc.	27
	3	60	1500 (+follicle stimulating hormone)	Subc.	19
	3	60	1500 (+pregnant mare serum)	I.P.	89
	3	—	— Control	—	18
Hypophysectomized immature female rats	10	50	1250	Subc.	11[2]
	10	100	2500	Subc.	7
	—	—	— Control	—	7
Immature male pigeons	4	100	2500	Subc.	27
	—	—	— Control	—	24

[1] Four types of tests were made in normal rats for possible gonadotrophic effects of lactogenic hormone preparations, i.e., a direct test for gonadotrophic activity; an augmentation test for FSH by injection of a mixture with human chorionic gonadotrophin (alone, ovaries 29 mg.); an augmentation test for the LH or ICSH by injection of a mixture with FSH (alone, ovaries 20 mg.); a test for antagonism of the action of other gonadotrophins (PMS) (alone, ovaries 99 mg.). Averages based on 3 animals per group.

[2] No repair of deficient IT, no follicular development.

that placentomata were produced by the administration of this hormone in hypophysectomized as well as in normal animals. Preparations containing 25 I.U. per mg. were effective in placentoma stimulation at 1 mg. daily doses; 0.5 mg. doses were only effective in part of the cases.[6] The latter dose was also consistently found to be the minimal effective dose in stopping estrous cycles of the normal adult.

Several of the lactogenic hormone preparations used were contaminated with small amounts of adrenocorticotrophic hormone. It was therefore considered important to test the effect of preparations more potent in adrenocorticotrophic hormone. These results are also given in table 5. As all the adrenocorticotrophic preparations employed were contaminated with lactogenic hormone (2.5 to 5 I.U. per mg.), the possibility remained that placentoma formation in such instances was due to contaminating lactogenic hormone. On the other hand, the injection of lactogenic hormone free of effect on the adrenal cortex, at dose levels in excess of those employed for placentoma

[6] This statement refers to the standard weight of animal used in these experiments, 140–180 gm. Younger animals (29 days of age, 65–80 gm. in weight, hypophysectomized 72 hr. after corpus luteum formation had been stimulated by injection of 15 R.U. of FSH over a period of 3 days) responded in every case with placentoma formation when given either 0.5 or 0.1 mg. of lactogenic hormone daily for the usual 10-day period with thread insertion on the 5th day and autopsy on the 11th day (6 rats in each group).

studies, enables us to say that lactogenic hormone preparations cannot be considered to stimulate placentoma formation because of their contamination with adrenocorticotrophic hormone (15). The other test of this point is not possible at present as the adrenocorticotrophic hormone is still in a relatively impure state. But it can be said that the lactogenic hormone does not act through stimulation of the adrenal cortex to secrete progesterone-like substances as the hormone was still effective in the stimulation of placentomata in doubly adrenalectomized rats (table 6). As the tumors were produced in hypophysectomized animals by injections of lactogenic hormone, it is not possible to regard the hormone as participating in this phenomenon through its influ-

TABLE 8. INFLUENCE OF POSTOPERATIVE PERIOD ON STIMULATION OF PLACENTOMATA BY LACTOGENIC HORMONE IN HYPOPHYSECTOMIZED RATS

Postoperative period			Duration of injection	Total dose[1] subc.		Placentomata		Ovaries	
						No. of rats			
Onset of injection	Threads inserted	Autopsy				Pos.	Neg.	Weight	Histology
days	*days*	*days*	*days*	*mg.*	I.U.			*mg.*	
0	4[2]	10	10	50	1250	4	0	33	Few l CL (cells large with abundant cytoplasm); no F growth; IT deficient
0	9	15	15	90	450	1	2	18	Positive case: few very large CL (large cells); no F dev.; IT def. Negative cases: same, except m CL
11	15	21	10	60	300	0	6	18	CL smaller (cells smaller, more connective tissue); no F dev.; IT deficient

[1] For discussion of freedom from contamination of these preparations see tables 5 and 7.
[2] Day of the 5th injection.

ence on the hypophysis.[7] Placentomata were not produced when lactogenic hormone was injected into gonadectomized animals (table 6), clearly indicating that the gonads are necessary for the effect and suggesting that the hormone acts through the ovary, yet table 7 shows the freedom of the lactogenic hormone from gonadotrophic hormones, so that it appears improbable that lactogenic hormone is effective in placentoma formation by virtue of its content of the gonadotrophic complex as usually understood. Nevertheless, it is probable that lactogenic hormone is effective in placentoma formation through its influence on the ovary, and possibly should be included as a member of the gonadotrophic complex.

Histological study of the ovaries of hypophysectomized rats injected with lactogenic hormone shows an effect on the ovary which may indicate its mode of action. Corpora lutea are present resembling those of pregnancy, follicular growth is suppressed and the 'deficiency condition' of the interstitial cells persists. The gonadal effect thus appears to be a specific one on the corpora lutea, for the ovarian follicles and the interstitial tissue are clearly uninfluenced, resembling these structures in hypophysectomized, untreated controls.

Inasmuch as it is well known that corpora lutea persist in an apparently healthy condition in the ovaries of hypophysectomized rats for many weeks, the reader might be inclined to ascribe the above-mentioned placentoma formation to the spontaneous

[7] It has been suggested by Riddle (16) that the lactogenic hormone could have its characteristic action on the estrous cycle of normal animals by suppressing the action of FSH on the part of the pituitary.

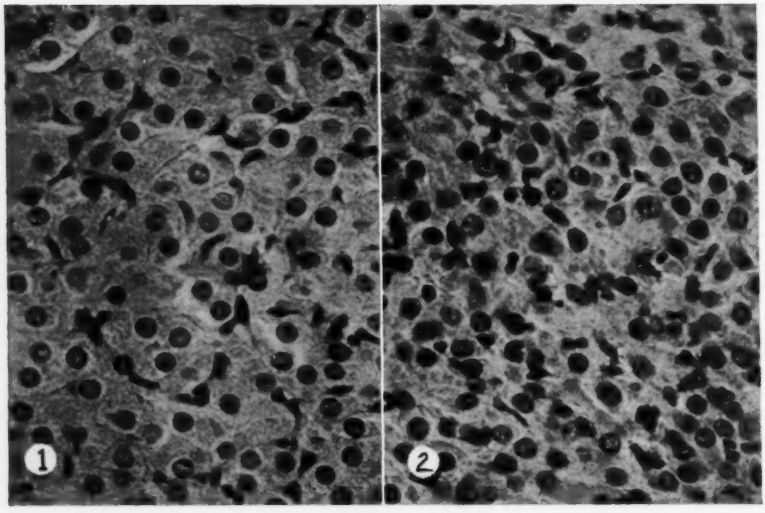

Fig. 1. Portion of a corpus luteum from the ovary of a rat hypophysectomized at 66 days of age. Subcutaneous injections of 1 mg. of lactogenic hormone given daily for 10 days, beginning the day of operation. Threads inserted in uterus on day 5; placentoma found at autopsy, day 11. The corpus luteum cells are larger, more uniform in size and more clearly outlined than the lutein cells of the control ovary. Fig. 2. Portion of a corpus luteum from the ovary of a control rat, hypophysectomized at 73 days of age. No injections given; threads inserted in uterus on day 5. No placentoma found at autopsy, day 11. (Hematoxylin and esosin; ×450.)

function of these persisting corpora lutea rather than to their stimulation by lactogenic hormone, but threads in the uteri of untreated hypophysectomized animals at the same postoperative period did not lead to the production of placentomata[8] (13 rats

Table 9. Stimulation of placentomata by lactogenic hormone in hypophysectomized rats in which corpus luteum formation had been induced by gonadotrophic hormone after a 10-day post-operative interval

Supplementary Therapy	Lactogenic hormone			Placentomata		Ovaries	
	Total dose in 10 days IP		Days Postop. on which administered	No. of rats			
				Pos.	Neg.	Weight	Histology
	mg.	I.U.				*mg.*	
None	50	500	10 to 20 (threads day 14)	0	3	25	Corpora degenerate; sF, IT degenerate
Gonadotrophic Hormone[1]	50	500	13 to 23 (threads day 17)	2	2	49 (pos.) 15 (neg.)	Ovaries of rats with placentomata contained 1 vascular CL, evidence of degeneration of the CL present in unreactive rats

[1] Pituitary FSH, 10 R.U., mixed in vitro with 1 R.U. chorionic gonadotrophin (antuitrin-S) injected subcutaneously daily for 3 days beginning 10 days postoperatively.

[8] E. B. Astwood and R. O. Greys (*Proc. Soc. Exper. Biol. & Med.* 38: 713. 1938) find that pseudopregnant rats when hypophysectomized are unable to produce placentomata. In the same paper he reports a luteotrophic substance prepared from rat placenta.

average weight of ovaries, 35 mg., see figure 2). Nor were they produced by the treatment of hypophysectomized rats with lactogenic hormone if the injections were not begun soon after operation (table 8). All positive results reported were secured in animals injected immediately after hypophysectomy (corpora lutea being present at time of operation). The insertion of threads could be delayed for 5 to 9 days after hypophysectomy if lactogenic hormone injections were begun the day of operation.

Placentomata could, however, be induced by lactogenic hormone in hypophysectomized rats after a 10-day period of regression provided a new crop of corpora lutea were induced by means of gonadotrophic hormone (table 9).

SUMMARY

The influence of various anterior pituitary hormones on placentoma production was studied by a standard technic in both normal and hypophysectomized rats. The effects of 'complete' gonadotrophic hormone as well as of various components of the gonadotrophic complex were studied. Complete gonadotrophic hormone from sheep pituitaries, and FSH contaminated with only small amounts of ICSH, occasionally caused placentoma formation in normal but not in hypophysectomized rats. Certain non-pituitary gonadotrophic preparations, even very potent ones, like that from the serum of pregnant mares, although creating abundant luteinization in the ovaries of hypophysectomized rats, were not able to bring about the conditions essential for the development of placentomata. Under the conditions of these experiments the principle in the urine of pregnant women was similarly ineffective.

The anterior pituitary lactogenic hormone favored the production of these tumors in normal, adrenalectomized and hypophysectomized animals but not in ovariectomized ones. The effect is probably a direct one on the ovary and on the corpora lutea of the ovary, a conception sustained by the histological finding that hypertrophic corpora lutea are present even in cases where both follicles and interstitial tissue are markedly subnormal. If the production of placentomata is à measure of corpus luteum function, the lactogenic hormone, not ordinarily considered as gonadotrophic in its effects, must be considered to awaken or intensify corpus luteum function.

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GROWTH OF THE ENDOCRINE GLANDS AND VISCERA IN THE CHICK[1,2]

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THE GROWTH AND DEVELOPMENT of the White Leghorn chick from hatching until 30 days of age is very rapid as was shown in previous work, Breneman (1). It was suggested (2), that a secretion of gonadotrophic hormone by the pituitary and androgenic substance by the testes may begin as early as the fifteenth day after hatching. No analysis has been made, however, of the secretion of other hormones nor of the development of the viscera during this period of rapid growth. The sensitivity of the Leghorn chick to slight variations in pituitary hormone level, as evidenced by testes growth or inhibition, and to varying amounts of androgenic hormones, as demonstrated by comb growth, makes this form especially valuable both as a test animal and for the study of normal development. It was hoped that the presence of pituitary thyrotrophic hormone might be detected because the thyroid gland of the chick is easily stimulated, Smelser (3). The nutritional demands of the animals during this time also focus attention to the possibility that prolactin may be secreted and might cause splanchnomegaly (4). These varied data should, therefore, make it possible to analyze many of the endocrine and non-endocrine factors which are operating during the growth and development of the chick.

MATERIALS AND METHODS

Single comb White Leghorn and Rhode Island Red pullets and cockerels were used in this work. The animals were reared in a battery brooder and mash was available at all times to the animals which are designated as "normal diet." "Limited diet" chicks received food only on alternate days but were permitted to eat at will when the mash was present. Feeding of older animals, however, was limited in some instances to only a few hours on the feeding days; these series are noted in a discussion of the tables. Previous work both from this laboratory and others (5 6), has emphasized that there is considerable variability in the chick and that data for organ weights should be corrected for a uniform body weight. The weights of all organs in the tables presented here are accordingly calculated as a percentage of body weight, and the actual length of the intestines is shown as well as the length per 100 gm. of body weight.

The animals were killed by cervical fracture, the crop removed and body weight taken. Combs were removed by cutting with scissors parallel to the head. The intestine was severed at the gizzard and cloaca and the caeca removed. Both length and

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weight of the intestine, therefore, are exclusive of the caeca. The lighter organs were weighed on a chainomatic balance and the heavier ones, on a torsion balance. Care was taken to perform the autopsy in such a way that drying of the tissues due to exposure to the air might be reduced to a minimum. There were 532 chicks used in this study.

RESULTS AND DISCUSSION

The data on the White Leghorn cockerels are summarized in table 1. Body weight in these birds increased about 30 gm. per 5-day period for the first 15 days. The figures for the 15 to 30-day interval, however, show that growth was much more rapid during this time with a considerable plateau at the 25th day of age. This has been noted before in other experiments and may represent some essential difference in physiology or merely improper handling of the birds with respect to numbers in cage and size of feeding apertures.

TABLE 1. WHITE LEGHORN MALES AND FEMALES, NORMAL DIET

No. of chicks	Age	Weight	Comb[1]	Gonad[1]	Adrenal[1]	Thyroid[1]	Panc.[1]	Liver[1]	Intestine	
									Length	Weight
	days	gm.							cm.	gm.
					Males					
28	5	49.1	0.0238	0.0214	0.0171	—	0.569	3.67	(123.8)[2] 60.7	4.33
32	10	76.8	0.0453	0.0254	0.0171	0.00706	0.586	3.35	(91.8) 70.5	5.90
18	15	106.0	0.109	0.0349	0.0149	0.00612	0.638	3.61	(78.1) 82.8	5.11
36	20	175.2	0.164	0.0291	0.0154	0.00532	0.489	2.89	(51.2) 90.0	4.00
29	25	190.7	0.166	0.0320	0.0162	0.00541	0.492	3.27	(47.9) 91.4	5.41
30	30	272.3	0.491	0.0316	0.0127	0.00513	0.413	3.21	(35.4) 96.5	4.11
					Females					
14	5	47.8	0.0235	0.0146	0.0187	—	0.442	4.34	(117.2) 56.0	4.19
14	10	57.8	0.0256	0.0230	0.0242	0.00969	0.680	3.87	(112.3) 64.9	5.87
19	15	95.4	0.0280	0.0255	0.0153	0.00662	0.678	3.48	(79.5) 75.8	5.30
12	20	135.2	0.0417	0.0316	0.0166	0.00763	0.602	3.61	(56.4) 76.3	3.57
12	25	212.6	0.0321	0.0279	0.0124	0.00589	0.468	2.82	(39.1) 83.2	3.10
14	30	266.6	0.0430	0.0264	0.0123	0.00401	0.417	2.76	(34.5) 92.1	3.64

[1] Values are average percentages of body weight.
[2] Figures in parentheses are average lengths per 100 gm. of body weight.

Comb growth in these chicks is very striking because there is an increase of more than 20 times in proportional size during the 30 days. Growth is apparent at the 10th

day and is very different from the female and the Rhode Island Red chicks in this respect. From the 5th to 10th day there is an increase of about 100% in proportional comb size. There is a plateau in comb growth at 25 days, perhaps correlated with the body weight plateau, but it will be noted that from the 25th to 30th day the comb increased 300%. It is obvious that a considerable amount of androgen must be formed in these animals and that this androgen is acting even before the 10th day of age.

The growth of the testes is significant in view of the comb increment, not only because the gonads must be considered the source of the androgen but also because they indicate that pituitary gonadotrophin is being secreted. The testes show a definite increase in size from the 5th to 15th day at which time they reach their maximum size in relation to body weight. The testes are heavier throughout the entire time range than they were on the 5th day and indicate that the pituitary is releasing gonad stimulating hormone or hormones. The marked growth during the early stages indicates that this secretion is probably being released as early as the 10th day and certainly is effective before the 15th day. Testes size increase, when compared with comb growth, demonstrates that the amount of testicular tissue is not necessarily proportional to the quantity of the androgen which is being secreted. This may be explained by a greater effectiveness of the androgenic hormone after a threshold has been reached since growth of the testis is not uniform for all tissues which constitute that organ. Absolute weight does not indicate actual secretory activity. The amount of seminiferous tubule area increases more rapidly than does that of the intertubular tissue and a direct correlation has been shown in the chick between this increase and the amount of androgen as demonstrated by comb growth.

Adrenal weights in the cockerels are surprisingly constant with only a slight indication of a drop in proportional size at the 30th day, which will be noted later. The observed variation in the series is not significant for the number of animals used and the question is immediately raised whether any adrenotrophic hormone is secreted by the chicks during this period. It will be noted that the adrenal weights in the female are similar.

If the weight of a gland is presumed to be indicative of its function it would have to be concluded that the thyroid is less active in the older chicks because the relative size is considerably less. No weights of the thyroids were taken on the 5th day in the Leghorns because of the difficulty of removing them cleanly and even identifying them in some instances, but from the 10th to 30th day there was a rather steady decrease in thyroid size. A cytological study of the thyroid gland is needed because of the very important part which the thyroid secretion must be playing in general metabolism. This is further emphasized by the fact that there is a very marked decrease in relative body surface and a change from down feathers to adult feathering during the interval, and these two factors must have considerable influence on heat loss and metabolism.

The growth of the viscera present some puzzling features which are not as evident in the cockerels as in the other groups. The pancreas has a maximum size at the 15th day and decreases after that time to a minimum on the 30th day. It is relatively smaller than at the 5th day. It should be noted that the maximum was reached at the time the most rapid rate of growth was just starting and also when the androgen was producing its greatest effect on the animals. The liver weights follow the same general trend as those of the pancreas, but extremes are less marked and perhaps would not be considered significant were it not for the greater differences observed in the other series. Maximum intestine weight occurs at the 10th day, which is 5 days earlier than the greatest weight for the pancreas and liver. After the 10th day there is considerable variation in its true weight but in general the trend is toward a proportionally

lighter alimentary tract. This decrease, however, must be considered along with the variation in intestine length and appears in a different light when the latter is considered. Actual length of the intestines is greater at each succeeding time period, but the length per 100 gm. of body weight is progressively less and at 30 days is less than one-third of the proportional length at 5 days. Since the percentage of weight does not differ appreciably on these two days it is obvious that the intestines must have increased greatly in diameter and in mass in order to offset the very marked diminution in relative length. A discussion of some of the factors which may be influencing the growth of the viscera will be considered with the data for the other birds.

The pullet data aid in the interpretation of several problems raised by a study of the cockerels. The female chicks grow somewhat more slowly than do the males and the body weight averages, as shown in table 1, are lower than the corresponding weights for the cockerels except on the 25th day. It was noted that perhaps the 25-day series is not typical in the males. The combs in the two series are almost identical on the 5th day but the growth rate in the females differs notably from the male. There is no appreciable growth before the 20th day which is 10 days later than that observed for the males, and by the 30th day the proportionate increase amounts to only double that of the 5th day which demonstrates a very considerable difference in the secretion of comb growth promoting substances in the two sexes. The combs are smaller, less turgid, and more deeply serrated than the cockerel combs and the possibility that this growth is due to estrogen secretion must be considered.

Gonad weight proportions are also lower in the females, but there is evidence of stimulation by gonadotrophic hormone. Again, as in the case of the comb, this increase occurs 5 days later than in the males with the maximum falling on the 20th day. The size is less on the subsequent days but is greater than that observed previous to the 20th day. The relative growth of the cortex and medulla of the ovaries has not been determined and such a study may make it possible to ascertain which region is the source of the comb growth promoting substance and perhaps whether an androgen or an estrogen is being secreted.

The data for the pullet adrenals are more variable than were those for the male glands, but there is no increase in proportional size. Actually, at the 25th and 30th days the adrenals are lower in relative weight than at any other time and this further emphasizes the observation that the adrenals are the lightest in cockerels on the 30th day. The weights of the female adrenals suggest to a lesser extent the phenomenon observed for the thyroids, that of a decrease in relative size with age.

The development of the thyroid gland occasioned some surprise in the males and the data for the females are even more notable. Whereas the decrease in relative thyroid size in the cockerels was about 70%, the decrease in the pullets was nearly 250%. The thyroids are proportionally much heavier than in the males on the 10th day but were lighter on the 30th. A very careful check of basal metabolism and of thyroid secretion is indicated as a future problem and is necessary before valid conclusion can be made regarding thyroid function in the chick.

The differential growth rate of the pancreas of the pullets is even more variable than in the cockerels. The maximum size, however, occurs 5 days earlier on the 10th day and does not decrease so rapidly but is almost identical with the male size on the 30th day. Liver weight is fairly uniform during the first 20 days after hatching but subsequently drops appreciably; the difference is much more marked than in the case of the males. Intestine length decreases in relative proportions as the animals become older and is, on the average, slightly less than the length in the cockerel. The weight maxima are found on the 10th and 15th days with a relative decrease after the latter day and it must be concluded that the factors operating in cockerel and pullet are

very similar as regards visceral growth. There is apparently a direct correlation between pancreas, liver and intestine weights.

The data for the Rhode Island Reds are much less extensive than that for the White Leghorns but there are definite differences to be observed between the two varieties. The results are shown in table 2 and demonstrate that this variety does not grow as rapidly as the Leghorn. There is a sex difference in growth rate and the males are consistently heavier as was the case with the Leghorns. Much more inter-

TABLE 2. RHODE ISLAND RED MALES AND FEMALES, NORMAL DIET

No. of Chicks	Age	Weight	Comb[1]	Gonad[1]	Adrenal[1]	Thyroid[1]	Panc.[1]	Liver	Intestine	
									Length	Weight
	days	*gm.*							*cm.*	*gm.*
					Males					
12	5	45.3	0.0171	0.0189	0.0184	0.00474	0.469	3.67	(123.0)[2] 55.7	3.79
12	10	82.5	0.0133	0.0256	0.0160	0.00482	0.644	3.77	(83.2) 68.7	4.50
11	15	103.0	0.0181	0.0193	0.0140	0.00684	0.551	3.45	(65.5) 67.4	4.11
12	20	162.0	0.0231	0.0208	0.0120	0.00657	0.505	3.17	(51.2) 84.8	3.90
—	25	—	—	—	—	—	—	—	—	—
13	30	214.6	0.0495	0.0168	0.00942	0.00508	0.469	2.76	(45.8) 98.1	4.28
					Females					
12	5	42.1	0.0218	0.0251	0.0251	0.00945	0.488	3.96	(119.0) 50.2	3.98
12	10	75.2	0.0155	0.0266	0.0191	0.00970	0.618	4.32	(85.1) 64.1	4.41
12	15	107.9	0.0152	0.0224	0.0124	0.00722	0.598	3.55	(64.9) 70.1	3.51
12	20	153.7	0.0175	0.0239	0.0137	0.00725	0.517	3.08	(66.3) 81.0	3.96
11	30	194.5	0.0177	0.427	0.0117	0.00658	0.535	2.89	(48.4) 94.2	4.08

[1] Values are average percentages of body weight.
[2] Figures in parentheses are average lengths per 100 gm. of body weight.

esting from the endocrine aspect are the differences observed in comb and gonad growth. The Rhode Island Red cockerels have combs on the 30th day which are only slightly larger in relative size than those observed for the 10-day-old White Leghorn cockerels and although there is a slight indication of comb stimulation on the 20th day, the proportional size is less than that of the 5-day-old Leghorn. There is, furthermore, no comb increment in the female which would indicate a secretion of any comb growth promoting substance.

Gonad weights fluctuate greatly in the cockerels but it is doubtful if the size increases are indicative of the secretion of pituitary gonadotrophin. Growth increase in the combs by the 30th day is certainly evidence of physiologically active testes, but the amount of pituitary secretion responsible for this is undoubtedly less and

formed later than in the White Leghorn males. There is stimulation of the ovary by the 30th day and it is actually relatively much larger than in the Leghorn pullets. This further emphasizes the need for a detailed study of ovarian development in the chick.

It was noted in the Leghorns that the adrenal gland decreased somewhat in relative size with age and this is significant for the Rhode Island Red series in which there is a 100% decrease in relative size in both male and female. Furthermore, this proportional decrease is uniform with only one slight variation on the 20th day in the pullets. There also seems to be a very definite tendency for the adrenals to be lighter in the males of the Rhode Island Reds, a condition which does not exist in the Leghorns. Additional animals are needed, however, to clarify this point.

Thyroid glands are relatively lighter at 30 days in the Red pullets than they were at 5 days and again are comparable to the White Leghorns. It should be noted that they are consistently heavier in percentage weight. The thyroids of the cockerels are not typical of any of the other series because they have maximum sizes on the 15th and 20th days, but they are relatively lighter again on the 30th. Allowance should be made in these results for probable error on the 5th day because of the difficulty in the removal of the thyroids at that time.

The weight maxima for the pancreas and liver are similar to those for the Leghorns but intestine weights are even more uniform and it must be concluded that despite variety differences in comb, gonad and adrenals, the viscera are very much alike in their growth responses. Intestine lengths decrease relative to body size throughout the series and are similar to the other data for the viscera.

The effect of inanition on the pituitary has recently been investigated rather extensively (6, 7), and it has been suggested that a pseudo-hypophysectomized condition is developed in animals by such procedure. It would seem profitable to compare the growth of the various organs in animals which are on a limited diet with the controls to see if any considerable change in pituitary secretion or organ weights can be detected. Body weights in the limited diet birds are considerably lower than the control weights after the first 10 days. The greatest difference is on the 20th day at which time the controls are more than 2.6 times heavier than the dieted animals and on the 30th day the normal chicks are 70% larger. It was during the period from the 10th to the 25th day that the food intake was curtailed the most because this appeared to be the most active period of development in the control chicks. The data show that under the conditions of this experiment hormone secretion is very definitely lower with respect to comb and testis growth but that it is by no means completely suppressed. The figures are summarized in table 3.

The increase in comb size from the 10th to 25th day was less than 100%. Although this is a significant increment, it is considerably less than that observed in the controls which have nearly a 400% increase in comb proportion. The fact that diet was not so rigidly controlled during the 25 to 30-day period is reflected in the body size increase. Weight increase at this time is the maximum recorded for the series and this in turn is accompanied by nearly a 300% increase in relative comb weight. It may be noted, however, that the size is only about 25% of that for the month-old control chicks.

There is only one very noticeable variation in testes proportion before the 30th day; this occurs at the 15th day and is correlated with the first definite stimulation of comb growth. The testes sizes are much smaller than those in the normal diet animals of the same age until the 30th day at which time there is no significant difference. It must be concluded that the secretion of pituitary gonadotrophin is less in the chicks placed on a limited diet but the animals cannot be considered to be pseudo-hypophys-

ectomized nor is there a complete cessation of androgen secretion from the testes.

The weights of the other organs contrast with the comb and testes inasmuch as they are heavier in relation to body weight. There is a significant increase in the percentage adrenal weight at all of the time periods except the 10th day but the proportional increase at no time is as great as 100%. The maximum difference is present on the 20th day but the actual weight does not exceed that of the control. An interpretation of these results can best be considered after reference to the data for viscera.

Pancreas weight, intestine length and weight and even liver weight are in general considerably heavier in proportion to body size than in the normal diet cockerels.

TABLE 3. WHITE LEGHORN MALES LIMITED DIET

No. of chicks	Age	Weight	Comb[1]	Gonad[1]	Adrenal[1]	Thyroid[1]	Panc.[1]	Liver[1]	Intestine	
									Length	Weight
	days	*gm.*							*cm.* (135.0)[2]	*gm.*
15	5	40.3	0.0241	0.0201	0.0221	—	0.506	3.72	54.4	3.23
									(124.1)	
25	10	51.5	0.0246	0.0197	0.0146	0.00268	0.593	4.00	63.9	4.93
									(120.9)	
31	15	61.2	0.0391	0.0260	0.0190	0.00431	0.656	3.74	74.0	6.01
									(119.6)	
27	20	66.1	0.0365	0.0191	0.0238	0.00499	0.710	4.23	79.1	6.44
									(93.1)	
30	25	95.5	0.0441	0.0186	0.0215	0.00551	0.611	4.12	88.9	6.18
									(57.6)	
27	30	158.9	0.148	0.0321	0.0176	0.00733	0.615	3.68	91.5	4.71

[1] Values are average percentages of body weight.
[2] Figures in parentheses are average lengths per 100 gm. of body weight.

This is especially true of the later time intervals from the 15th to 25th day when the food limitation was most acute. The actual weights again do not exceed those of the control but only the percentage weights. The weight of the pancreas differs most notably and the intestine length is surprisingly similar to that of the normal diet animals in spite of the great difference in body size. It had been expected that the liver would be small because there would seen to be little possibility for storage of foodstuff, but it is obvious that at every period the liver is relatively as heavy or heavier than in those chicks which received ample food.

These results naturally require explanation and would indicate that the visceral growth is somewhat independent of the body growth and possibly is under pituitary stimulation even in the limited diet birds. It has been suggested by Schooley (8) that the secretion of prolactin, which has such a pronounced effect on the growth of the viscera, may be elaborated by the eosiniphils of the pituitary and the gonadotrophin by the basophils. This speculation may throw considerable light on the problem.

A very extensive study has been made in the last few years of the chick pituitary by Dr. Fernandus Payne of this laboratory and he has observed (unpublished data) that there is an almost complete disappearance of basophils in the anterior lobe of the pituitary of the limited diet chick, but that the eosinophils are only slightly affected. The anterior lobe is likewise somewhat smaller. If, therefore, the suggestion of the Riddle group is correct, the observed variation in the visceral weights has a very log-

ical explanation. The almost complete loss of basophils would account for the marked diminution in percentage of testes weight due to insufficient secretion of gonad stimulating hormone, and indirectly the smaller combs would reflect the low level of testicular androgen secretion. The maintenance of the visceral weights and their proportionally larger size would be explainable because the limitation of diet has had little effect on the numbers of eosinophils and their secretion, presumably of prolactin.

The thyroid weight variations in the limited diet series are more difficult to interpret. The very low weight at 10 days perhaps indicates a cessation of thyrotrophic hormone secretion. With increasing age, however, the thyroid weights increase and are essentially normal for body weight on the 20th and 25th days. The problem is further complicated by the fact that the relative thyroid weight on the 30th day exceeds that of the control by a significant amount. It would be premature to postulate that the glands are more active because an increase in size may be merely an expression of hypothyroid condition. It should be possible by a cytological study of the glands to determine the physiological state of the thyroid.

The effects of malnutrition reported by Mulinos and Pomeratz in the rat are interesting because they show some dissimilarities when compared with the chick. Complete starvation in the rat produced a decrease in liver weight greater than the proportionate body decrease but chronic inanition had much less effect. These results would not correspond to the chick data. Likewise the adrenal weights were lower in 'chronic' malnutrition whereas in the chicks they were heavier, but the adrenals of the 'complete' group were heavier, thus corresponding to the chicks. These differences and others which are apparent from their data demonstrated clearly that the problem is a very complex one. A very thorough analysis of pituitary cytology and function is needed and an equally careful analysis of the study of the thyroid and adrenal. The necessity for the maintenance of a normal metabolic rate in spite of the very great modification of growth rate places a demand on the organism which requires complex readjustments and speculation concerning the mechanism of this adjustment should be cautious. Species differences as in rat and chick are indicated, and, in addition, variety differences in the White Leghorns and Rhode Island Reds must be considered with allowance for the sex of the animals.

Some workers have used chicks from mixed breeds for endocrine experimentation. Such a practice will undoubtedly lead to erroneous interpretations of results because of the very great divergence in the normal development of the different varieties. The differences noted between normal and limited diet chicks emphasize that care should be taken to rigidly control diet conditions. An unwillingness on the part of some investigators to use chicks as experimental animals can probably be traced to variation which has been the result of a failure to take the two preceding facts into consideration.

SUMMARY

A study was made of the growth of single comb White Leghorn and Rhode Island Red chicks from hatching until 30 days of age. The following weights were recorded for both cockerels and pullets: body (less crop), comb, gonad, adrenal, thyroid, pancreas, liver and intestine (empty). Intestine length was also measured. All of these weights were tabulated as a percentage of body weight and intestine length on the basis of 100 gm. of body weight. A series of White Leghorn cockerels was also used in which the diet was limited to approximately one-half the normal food intake, and the same weights and measurements were taken. A total of 532 chicks was studied.

The White Leghorn chicks grew more rapidly than did the Rhode Island Reds and the weight of the cockerels of both groups exceeded the pullets slightly. The in-

crease in body weight in the Leghorn cockerels was about 525% from the 5th to the 30th day; for the Red cockerels about 480%. The comb in the White Leghorn males increased 20 times in proportion to body weight and there was definite evidence of gonad stimulation as early as the 10th day of age indicating that the pituitary gland was secreting appreciable amounts of gonadotrophic hormone early. Androgen was being released at this time also. The pullets showed much less comb growth and gonad stimulation, and response occurred 5 to 10 days later than in the males. Adrenal glands were of a uniform proportion throughout the series with some evidence of a decrease in relative size at the 30th day. The thyroid glands of both sexes, however, decreased in relative size with age but this was much more marked in the pullets. Viscera had a maximum weight at about 15 days, and the intestines decreased in relative length with increasing age, but the weight was fairly constant.

The comb and gonad growth was very much slower in the Rhode Island Red chicks but there was evidence of pituitary, testicular and ovarian secretion before the 30th day. The weights of the other organs were similar to those of the Leghorns but with the difference in adrenal weight at the 30th day being even more notable.

It was possible to inhibit the growth of the White Leghorn cockerels very markedly by limiting the diet and to suppress but not eliminate the secretion of gonad stimulating hormone from the pituitary. Comb growth increased about six times in proportion to body size with half of this increase occurring at the 25th to 30th day interval when diet was less restricted. The adrenal glands were heavier and the thyroids increased in weight with age. This was the reverse of the normal growth picture. Visceral weights were heavier than in the control diet chicks and a possible explanation for this on the basis of pituitary cytology was advanced.

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FINDINGS IN 216 ROUTINE AUTOPSIES OF *MACACA MULATTA*[1]

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DURING recent years the increasing use of non-human primates for experimental purposes has brought to light a vast amount of material on various phases of their life in health and disease. We have now learned through experience that these animals in captivity may be maintained in an excellent state of health and nutrition. Breeding has become possible. Data concerning their normal age span, weight, and developmental relationships become essential.

For this reason, two years ago, routine consecutive autopsies upon all primate forms dying in the Laboratory of Physiology of the Yale School of Medicine were undertaken by the authors, and the material from this study, together with data previously collected in the same Laboratory are here published in a series of three papers dealing with the findings at autopsy of (*a*) the present group of 216 *Macaca mulatta* (*Macacus rhesus* of the older terminology) (*b*) 70 anthropoid apes (1), (*c*) 132 monkeys of miscellaneous species (2). The names of species used here are given in accordance with the nomenclature of Zuckerman and Fulton (3). A report of diseases encountered in these animals will be reported elsewhere (4).

The literature on the anatomy of the non-human primates is not large. It can be divided into two parts: that dealing with disease, which is reported elsewhere (4) and that concerned with the growth, and weight of various species. Body measurements, skeletal development and dentition especially of the anthropoids are described by Schultz (5, 6) and "The anatomy of the rhesus monkey" (7) compiled under the direction of Hartman and Strauss contains valuable information. Kohlbrugge (8) has reported a large series of organ weights of *Semnopitheque*, and Hrdlička (9) of New and Old World monkeys of various species. Inay, Ruch, Finan and Fulton (10) have given data concerning a series of primate organ weights from this Laboratory.

Data concerning the weights of brains of various species is plentiful. Keith's series (11) reported in 1895 is one of the largest; that of Hrdlička (9) includes both New and Old World monkeys; Zuckerman and Fisher (12) have correlated dentition with body and brain weights; von Bonin (13) includes primates in his mammalian brain weights; and the series of Marquis reported from this Laboratory (Fulton and Keller, 14) presents another group of data.

METHOD

In the two years during which this material was collected, autopsies were performed as routine on all monkeys and anthropoids dying in the Laboratory. There were 216 autopsies on *Macaca mulatta*, of which 118 were complete, including weighing and gross examination of all organs. The remaining 98 were incomplete because at the time of examination it was felt that thorough autopsy and more particularly weighing of organs would be useless because of the condition of the carcass at the

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time. This was due in 64 instances to gross disease and in 34 instances to the fact that the death of the animal occurred at a time when immediate examination was impossible (table 1).

The monkeys had been in the Laboratory for a length of time varying from a few days to several years. Most were bought directly from dealers and for the most part had been obtained recently from India. A few had been born in the Laboratory. They had all lived under uniform conditions of diet, housing and environmental temperature. Nearly all were used prior to autopsy in some phase of research on the central nervous system.

All autopsy examinations were made by one or both authors. A routine procedure was carried out which began with general inspection of the animal and recording of the sex, approximate age, the nutritional state, and the condition of the hair, nails and skin. Dentition was then examined. There followed a detailed examination of the cen-

TABLE 1. A TOTAL OF 216 AUTOPSIES ON MACACA MULATTA GROUPED INTO 98 INCOMPLETE AUTOPSIES AND 118 COMPLETE AUTOPSIES

Total autopsies	216
1. *Complete autopsies*	118
A. Rated as 'healthy'	66
B. Rated as 'unhealthy' because of:	52
Bronchopneumonia	1
Tuberculosis	6
Intestinal parasitism	21
Gastric ulcer	1
Post-operation	5
Experimental procedure	13
Prolonged anesthesia (dehydration)	4
Unknown (loss of weight)	1
2. *Incomplete autopsies*	98
Because of: Tuberculosis	36
Intestinal parasitism	7
Pneumonia	11
Experimental procedure	44

tral nervous system. The pituitary, the thyroid, and the contents of the thoracic and abdominal cavities were examined in that order. The deposition of fat and the condition of the lymph nodes were noted.

Additional facts recorded at autopsy as of possible significance were: length of time in the laboratory, weight on arrival, maximum weight and autopsy weight; date and character of operations; and date and hour of death (approximate in some cases); hour and date of autopsy examination; and whether or not tissue had been injected with formalin at time of death. All animals were routinely fed between 1:30 and 3 P.M. daily, so that time of death after feeding could also be calculated. If anesthetic had been used preceding death, this was recorded also. A summary of significant findings, disease, etc. was noted at the end of each record.

Weighing was done on a balance, accurate to 0.01 gm. except in the case of the parts over 100 gm. which were weighed on a less sensitive scale. Histological examination of portions of organs was made whenever indicated.

The usual procedure when an animal was killed was to anesthetize the animal and then to inject a liter of normal saline followed by the same amount of 10% formalin into the left ventricle of the heart. This was done under gravity pressure of about 150

cm. The right auricle was slit to allow escape of fluid. Many animals succumbing for other reasons were injected in the same way.

Grouping. Since it was the object of this study to establish normal values and to discover deviations from the normal in a series of routine autopsies, various arrangements of the data have been tried and many discarded as of no significance. For purposes of statistical analysis final groupings according to sex, weight and health were made (table 2).

1. *Age.* Little can be deduced about the age of animals because there were only 5 of known age in the group, and, although a record was kept of dentition, it was ultimately decided that it could not assist in determining age, because there were such wide variations in weight—dentition correlation. Also, with the exception of the small series of Zuckerman and Fisher (12), there are now no published standards of

TABLE 2. COMPLETE AUTOPSIES (118) GROUPED ACCORDING TO SEX, WEIGHT AND HEALTH

Group	'Healthy'	'Unhealthy'	Total
Infants (to 1,000 gm.)			
Male	2	0	2
Female	3	0	3
Total	5	0	5
Immature (1,000 to 4,500 gm.)			
Male	28	24	52
Female	28	23	51
Total	56	47	103
Mature (above 4,500 gm.)			
Male	2	2	4
Female	3	3	6
Total	5	5	10
Total	66	52	118

age and dentition of the *Macaca mulatta* which would assist in determining the approximate age of the animals.

2. *Sex.* The individuals have been grouped according to sex (table 2).

3. *Weight.* Three weight groups were formed which roughly correspond to 3 ages. The 'infant' *Macaca mulatta* were those under 1,000 gm. in weight. This should include animals up to about 5 or 6 months of age at which time they usually attain a weight of 1,000 gm. The 'immature' animals were those between 1,000 and including 4,500 gm. These limits were chosen because the females usually begin to menstruate at about 4,000 gm. and can be bred shortly thereafter (weight of 4,500 gm. or more). The testicles of the males enlarge rapidly when they attain a body weight of about 4,500 gm. The smallest fertile male in this colony during the past 10 years weighed 5,600 gm. and the largest, 9,800. The smallest pregnant female (before pregnancy) weighed 4,500 gm. and the largest (extremely fat) 7,300 gm. By this weight grouping there are 5 infants, 103 immature animals and 10 mature animals in the series (table 2)

4. *Health.* As these rhesus had been used in many types of experiment previous to death, numbers of them were not in the best of health and had lost weight because of some operative or experimental procedure. Some also had disease (table 1). They were therefore, divided into 'healthy' and 'unhealthy' groups. The first contained 66 indi-

viduals, none of which had lost more than 100 gm. weight at autopsy as compared to the maximum weight, and in none of which was disease found. The second, or 'unhealthy' group contained 52 animals each of which had either lost more than 100 gm.

TABLE 3. BODY AND BRAIN WEIGHTS OF 'HEALTHY' MACACA MULATTA

Males				Females			
No.	Body wt.	Brain wt.	Ratio	No.	Body wt.	Brain wt.	Ratio
	gm.	gm.			gm.	gm.	
1243	475	59.40	12.50	1445	500[1]	55.00	11.00
1448	495	47.91	9.68	1248	505	51.86	10.27
1435	1,160	75.72	6.52	1249	530	55.97	10.50
1157	1,850[1]	72.00	3.89	1124	1,050[1]	64.75	6.16
1321	1,900[1]	65.00	3.42	1253	2,040[1]	69.40	3.40
1319	2,050[1]	98.71	4.81	1051	2,200	68.00	3.00
1169	2,300	69.00	3.00	1293	2,200[1]	76.28	3.46
1192	2,400	86.12	3.58	1318	2,300	71.08	3.09
1290	2,500[1]	84.00	3.36	1287	2,300[1]	73.32	3.18
1288	2,500[1]	83.00	3.32	1159	2,400[1]	65.00	2.70
1285	2,540[1]	79.50	3.13	1338	2,500[1]	80.47	3.21
1365	2,550[1]	72.00	2.82	1130	2,700[1]	64.00	2.74
1257	2,650[1]	86.80	3.27	1262	2,700[1]	95.00	3.51
1323	2,650	83.82	3.16	1112	2,800[1]	75.00	2.67
1011	2,800	87.45	3.12	1034	3,000	80.97	2.76
1174	3,100[1]	90.00	2.90	1036	3,100[1]	57.60	1.80
1331	3,200[1]	94.17	2.94	1337	3,100[1]	78.48	2.52
1176	3,250[1]	82.00	2.52	1389	3,300	88.50	2.68
1376	3,300	83.40	2.52	1073	3,500[1]	92.16	2.63
1366	3,500[1]	91.00	2.60	1225	3,500[1]	82.75	2.36
1320	3,500	84.86	2.42	1260	3,600[1]	72.54	2.01
1377	3,600	96.00	2.66	1361	3,600[1]	69.80	1.93
981	3,700[1]	90.00	2.43	1114	3,650[1]	77.65	2.12
1317	3,850	79.50	2.06	1149	3,800[1]	80.00	2.10
1052	4,000[1]	96.70	2.41	1259	4,000[1]	80.45	2.01
1375	4,150[1]	93.33	2.25	1175	4,400[1]	85.46	1.94
1280	4,400[1]	110.00	2.50	1362	4,500[1]	80.02	1.77
996	4,500	95.06	2.11	1300	6,000[1]	92.23	1.53
922	6,400	86.21	1.35				
418	6,800[1]	110.00	1.62				

[1] Injected with 10% formalin at autopsy.

TABLE 4. ENDOCRINE WEIGHTS OF MACACA MULATTA

No.	Body wt.	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Gonads	
						Rt.	Lt.	Rt.	Lt.
'Healthy' Females									
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1445	500	0.03	0.31	0.42	0.51	0.37	0.48	0.09	0.09
1248	505	0.03	0.33	0.58	0.61	0.41	0.52	0.06	0.06
1249	530	0.16	0.22	0.15	0.57	0.30	0.32	0.11	0.12
1124	1,050	0.16	0.10	1.08	1.15	0.31	0.30	0.03	0.03
1253	2,040	0.04	0.26	3.49	5.54	0.22	0.41	0.06	0.08
1051	2,200	0.04	0.30	6.03	4.76	0.61	0.81	0.08	0.08
1293	2,200	0.01	0.42	1.71	6.05	0.18	0.25	0.06	0.05
1318	2,300	0.03	0.30	2.68	4.10	0.21	0.30	0.04	0.03
1287	2,300	0.04	0.28	1.35	6.37	0.21	0.22	0.02	0.02
1159	2,400	0.09	0.52	0.51	7.00	0.98	0.97	0.15	0.17
1116	2,500	—	—	2.80	6.71	0.72	0.90	0.28	0.27

TABLE 4—*Continued*

No.	Body wt.	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Gonads	
						Rt.	Lt.	Rt.	Lt.
				'Healthy' Males					
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1336	2,500	0.04	—	1.13	3.50	0.23	0.32	0.06	0.07
1338	2,500	0.07	0.28	1.40	5.30	0.35	0.37	0.05	0.05
1413	2,600	0.03	0.25	2.43	3.84	0.24	0.32	0.10	0.11
1130	2,700	0.06	0.75	6.20	6.10	0.59	0.59	0.12	0.14
1262	2,700	0.04	0.35	5.89	6.03	0.40	0.45	0.01	0.01
1112	2,800	—	0.81	1.03	—	0.80	0.74	0.09	0.10
1407	2,800	—	0.48	3.42	3.44	0.25	0.39	0.08	0.08
1034	3,000	0.20	0.42	7.60	—	0.60	0.68	0.35	0.30
1036	3,100	0.05	0.32	3.53	—	0.79	0.84	0.12	0.15
1337	3,100	0.04	0.58	8.13	8.76	0.24	0.31	0.12	0.15
1389	3,300	0.04	0.22	0.64	5.96	0.27	0.25	0.08	0.06
1073	3,500	0.03	0.31	4.73	5.30	0.42	0.55	—	—
1225	3,500	0.09	0.61	0.75	10.45	0.60	0.58	0.25	0.23
1260	3,600	0.03	0.58	9.65	8.23	0.22	0.29	0.04	0.04
1361	3,600	0.05	0.48	2.55	8.31	0.29	0.39	0.05	0.06
1114	3,650	0.05	0.82	1.00	—	0.36	0.29	0.07	0.05
1385	3,700	0.08	0.58	4.55	8.47	0.36	0.45	0.19	0.25
1149	3,800	0.05	0.56	0.61	—	1.00	1.06	0.31	0.23
1298	4,000	0.13	1.32	—	4.45	0.52	—	0.22	0.24
1259	4,000	0.07	0.72	3.92	6.02	0.40	0.54	0.18	0.20
1175	4,400	0.10	0.61	7.35	11.86	0.44	0.52	0.22	0.17
1362	4,500	0.10	0.55	6.58	11.40	0.45	0.58	1.40	1.40
1300	6,000	0.09	0.85	0.57	14.50	0.51	0.62	0.16	0.28
				'Healthy' Males					
1243	475	0.02	0.12	0.28	0.18	0.18	0.20	0.07	0.08
1448	495	0.02	0.21	0.71	0.42	0.37	0.40	0.14	0.16
1435	1,160	0.03	0.30	1.31	1.35	0.19	0.27	0.14	0.14
1157	1,850	0.04	0.26	2.67	3.91	0.11	0.20	0.21	0.13
1321	1,900	0.04	0.32	2.58	5.69	0.28	0.34	0.20	0.19
1319	2,050	0.12	0.43	4.37	4.75	0.19	0.24	0.30	0.32
1169	2,300	0.03	0.20	2.40	4.70	0.60	0.55	0.51	0.48
1192	2,400	0.16	0.65	3.12	5.41	0.47	0.55	0.41	0.43
1290	2,500	0.04	0.32	3.68	3.73	0.27	0.43	0.31	0.33
1288	2,500	0.04	0.39	0.23	4.68	0.32	0.46	0.30	0.28
1285	2,540	0.04	0.29	1.28	5.15	0.44	0.39	0.15	0.17
1365	2,550	0.04	0.32	1.38	3.45	0.20	0.26	0.20	0.17
1257	2,650	0.06	0.47	2.93	9.12	0.21	0.34	0.34	0.34
1323	2,650	0.09	0.35	2.18	4.10	0.26	0.35	0.21	0.21
1011	2,800	0.20	—	0.92	7.25	0.55	0.50	0.20	0.25
1174	3,100	0.06	0.91	3.83	7.05	0.90	1.20	0.71	0.70
1331	3,200	0.08	0.41	3.70	7.64	0.16	0.24	0.60	0.53
1176	3,250	0.11	0.51	0.75	9.35	1.05	1.22	0.35	0.30
1376	3,300	0.06	0.64	5.50	6.85	0.29	0.37	0.51	0.53
1367	3,500	0.05	0.67	9.83	6.86	0.22	0.34	0.64	0.55
1366	3,500	0.05	0.83	2.78	5.12	0.38	0.38	0.24	0.24
1320	3,500	0.06	0.26	Not found	3.62	0.53	0.59	6.04	5.56
1377	3,600	0.04	0.40	4.81	4.67	0.40	0.51	0.40	0.37
1339	3,700	0.04	0.24	4.15	5.78	0.40	0.43	0.58	0.58
981	3,700	0.10	0.68	11.43	8.42	0.64	0.55	0.87	0.79
1317	3,850	0.06	0.47	2.15	4.22	0.45	0.54	0.97	1.04
1052	4,000	0.08	0.74	4.76	11.08	0.44	0.57	1.49	1.36
1375	4,150	0.05	0.49	6.05	6.50	0.26	0.34	1.36	1.38
1280	4,400	0.10	0.70	2.00	9.94	0.88	1.10	1.13	1.11
996	4,500	0.08	0.50	1.89	8.12	0.27	0.35	8.27	5.90
922	6,400	0.12	0.42	4.94	—	0.50	0.88	6.06	5.26
418	6,800	0.04	0.63	0.53	8.40	1.01	0.81	11.81	11.21

TABLE 5. ORGAN WEIGHTS OF MACACA MULATTA

No.	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Rt.	Lt.				Rt.	Lt.
				'Healthy' Females				
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1445	500	—	—	3.75	18.61	0.64	1.84	1.86
1248	505[1]	4.72	3.73	2.58	21.03	1.51	2.36	2.70
1249	530	3.02	2.60	2.22	13.82	0.74	1.61	1.58
1124	1,050[1]	3.00	4.50	7.50	45.00	1.52	5.00	4.00
1253	2,040[1]	12.04	9.70	11.45	70.83	3.03	7.15	6.89
1051	2,200	10.51	8.40	12.64	65.41	3.20	6.07	5.31
1293	2,200[1]	15.37	11.28	11.09	64.78	5.91	7.18	7.39
1318	2,300	8.70	9.75	14.88	67.75	3.21	4.68	4.61
1287	2,300[1]	9.36	7.24	10.14	68.64	4.19	6.45	6.46
1159	2,400[1]	17.15	14.45	11.60	70.50	3.40	8.60	8.65
1116	2,500	8.80	7.00	14.13	83.73	5.10	6.15	6.38
1336	2,500	10.06	7.89	12.21	62.42	1.86	6.11	6.26
1338	2,500[1]	10.41	7.29	12.12	72.34	2.36	7.22	7.66
1413	2,600	9.48	8.83	10.43	67.52	4.08	5.49	5.27
1130	2,700[1]	13.88	12.41	12.38	71.74	3.72	8.72	8.80
1262	2,700[1]	18.85	14.95	12.78	70.78	6.65	7.28	7.60
1407	2,800[1]	12.70	13.07	11.38	93.31	3.86	6.93	7.49
1034	3,000	9.00	7.55	15.35	73.70	4.10	6.55	6.93
1337	3,100[1]	18.90	16.55	11.87	85.55	7.61	8.30	8.62
1389	3,300	8.86	11.52	14.79	99.18	3.40	8.12	8.26
1073	3,500[1]	9.00	10.00	15.00	70.00	3.86	7.48	7.54
1225	3,500[1]	8.82	10.55	16.05	112.50	5.70	7.72	10.75
1260	3,600[1]	22.00	19.06	16.10	85.10	6.28	7.86	8.93
1361	3,600[1]	20.32	16.84	16.06	111.00	3.87	10.34	9.92
1385	3,700[1]	15.05	11.61	17.85	103.87	4.91	9.49	9.64
1298	4,000	14.30	12.90	16.80	94.88	1.88	7.58	8.58
1259	4,000[1]	13.00	15.60	18.70	95.22	4.58	10.01	8.95
1175	4,400[1]	—	—	23.48	98.00	6.08	10.25	11.34
1362	4,500[1]	—	—	20.05	100.10	7.72	10.16	10.32
1300	6,000[1]	26.30	17.88	24.62	210.00	2.65	12.34	14.10
				'Healthy' Males				
1243	475	3.68	2.55	2.03	12.62	0.55	1.02	1.05
1448	495	2.73	2.33	3.21	19.38	0.59	1.25	1.24
1435	1,160	5.46	5.60	7.73	36.24	2.04	2.49	2.84
1157	1,850[1]	4.94	5.35	6.50	40.42	3.32	3.88	3.87
1321	1,900[1]	5.84	5.81	7.82	65.64	2.00	6.00	4.76
1319	2,050[1]	15.26	10.58	9.77	60.25	4.08	5.68	5.35
1169	2,300	13.80	20.00	11.00	71.00	3.20	6.10	6.20
1192	2,400	14.18	8.84	14.88	75.28	2.68	5.78	6.25
1290	2,500[1]	8.54	6.40	10.35	60.13	3.76	5.46	5.04
1288	2,500[1]	11.62	10.40	12.52	59.52	1.93	5.91	6.28
1285	2,540[1]	7.95	5.88	10.78	72.34	3.45	4.86	5.31
1365	2,550[1]	8.13	6.48	10.07	72.02	2.12	6.15	5.91
1257	2,650[1]	—	—	15.77	81.17	3.47	6.12	6.00
1323	2,650	8.51	8.35	12.88	62.36	2.55	6.36	6.44
1011	2,800	8.00	10.30	12.70	93.00	4.60	8.25	8.00
1174	3,100[1]	—	—	14.17	77.20	6.13	8.19	7.85
1331	3,200[1]	15.04	11.69	15.27	87.82	3.85	7.70	7.42
1176	3,250[1]	20.45	17.30	12.40	99.80	4.85	8.50	8.90
1376	3,300	9.93	10.20	18.33	69.66	3.32	6.26	6.19
1367	3,500[1]	15.20	11.84	16.65	77.25	4.84	7.74	7.46
1366	3,500[1]	—	—	13.44	92.22	2.32	8.64	8.78
1320	3,500	8.32	7.17	11.65	96.09	2.16	9.58	9.64
1377	3,600	10.15	10.88	17.91	87.62	2.66	7.24	7.62
1339	3,700[1]	14.20	8.10	19.46	103.94	3.70	9.15	9.50

[1] Injected with 10% formalin at autopsy.

TABLE 5—*Continued*

No.	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Rt.	Lt.				Rt.	Lt.
				'Healthy' Males				
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
981	3,700[1]	22.47	18.03	15.94	105.50	5.87	10.71	11.10
1317	3,850	12.45	10.32	14.54	114.00	3.26	8.84	8.93
1052	4,000[1]	15.82	14.27	21.19	95.16	5.89	10.21	10.23
1375	4,150[1]	—	—	16.44	120.00	4.98	13.02	12.33
1280	4,400[1]	25.93	23.20	23.32	118.00	6.08	11.16	12.15
996	4,500	17.61	13.07	15.48	94.38	8.61	6.31	6.63
922	6,400	17.95	14.65	20.05	115.00	6.26	10.32	10.28
418	6,800[1]	26.05	18.30	28.60	128.00	6.22	14.10	11.40

[1] Injected with 10% formalin at autopsy.

weight before autopsy, or had shown disease present at autopsy (table 1). However, this 'unhealthy' group was only relatively so. All monkeys showing severe disease in any form were not thoroughly examined but were included in the group of 98 incomplete autopsies mentioned above. No animals in the 'unhealthy' group were considered in poor health before death, and none showed either advanced disease or extreme emaciation at the time of death.

5. *Injection with formalin.* It was thought that injection with formalin might alter some of the weight values of the organs. However, when the two groups were compared, no significant differences were found except in the lungs, which will be discussed later.

6. *Anesthesia.* Similarly, there were no significant differences as a result of prolonged use of anesthetics immediately before death. A number of these animals which were kept under barbiturate anesthesia for more than 24 hours prior to autopsy, were, however, placed in the 'unhealthy' group because it was thought that dehydration might have affected organ weights.

DATA

In tables 3 to 5 the individual weights of organs of the 66 'healthy' animals are arranged according to ascending body weights. The actual weights of the individuals in the 'unhealthy' group are not published because of lack of space. The mean weights of the four groups,—males, females, 'healthy' and 'unhealthy,' together with the ratios of organ weight to body weight at autopsy, the standard deviation and the probable error are given in table 6.

Individual Organs

Individual organs were usually weighed intact and then sectioned for examination.

The brain was cut from the cord just below the pyramidal decussation. In some instances when cortical ablations had been made previously, the weight of the extirpated tissue was added to that of the weight at autopsy. These ablated blocks were usually not more than 1 to 2 gm. and never more than 5 gm. in weight. The condition of dura was noted and cerebral edema or infection whenever present. There is little else to be said concerning the central nervous system beyond the weights recorded in the tables.

The pituitary was removed intact and weighed. At no time either in this series or in those of the other primates was any gross abnormality noted. The gland apparently increases in size with the growth of the sella turcica and relatively little as compared to other organs.

The thyroid varied considerably in size and weight.

Parathyroids were not removed before weighing the thyroid. In nearly every in-

TABLE 6. MEAN WEIGHTS OF MACACA MULATTA BETWEEN 1,000 AND 4,500 GM. BODY WEIGHT

Organ	Mean wt., gm.	Ratio mean organ to mean autopsy wt.	σ[1]	P.E.M.[2]	Mean wt., gm.	Ratio mean organ to mean autopsy wt.	σ[1]	P.E.M.[2]
	'Healthy' females				*'Unhealthy' females*			
Body weight	2,923		711.3	90.69	2,122		593.49	83.40
Brain	76.55	2.62	8.849	1.24	74.53	3.51	7.666	1.16
Right lung	12.403	0.42	4.541	0.64	11.298	0.53	4.749	0.70
Left lung	11.132	0.38	3.664	0.52	9.903	0.47	4.558	0.67
Heart	13.749	0.47	3.273	0.45	11.065	0.52	2.787	0.40
Liver	79.54	2.72	16.697	2.30	68.808	3.24	15.306	2.20
Spleen	4.27	0.15	1.490	0.21	2.688	0.13	1.081	0.16
Pancreas	6.228	0.21	2.341	0.33	4.765	0.22	1.818	0.26
Right kidney	7.465	0.26	1.534	0.21	6.441	0.30	1.500	0.22
Left kidney	7.652	0.26	1.816	0.25	6.391	0.30	1.457	0.21
Pituitary	0.060	0.0021	0.042	0.0056	0.083	0.0039	0.071	0.01
Thyroid	0.458	0.016	0.191	0.025	0.456	0.022	0.184	0.026
Thymus	3.434	0.12	2.573	0.33	1.268	0.060	1.137	0.16
Right adrenal	0.439	0.015	0.236	0.030	0.446	0.021	0.123	0.017
Left adrenal	0.503	0.017	0.235	0.030	0.492	0.023	0.132	0.019
Both adrenals	0.941	0.032	0.466	0.059	0.938	0.044	0.247	0.035
Right ovary	0.119	0.0041	0.906	0.12	0.102	0.0048	0.053	0.0076
Left ovary	0.117	0.0040	0.830	0.11	0.097	0.0046	0.055	0.0080
	'Healthy' males				*'Unhealthy' males*			
Body weight	3,039		811.14	103.42	2,352		574.15	79.03
Brain	85.736	2.82	9.937	1.31	81.57	3.47	12.156	1.93
Right lung	12.492	0.41	5.331	0.73	10.30	0.44	3.756	0.55
Left lung	10.836	0.36	4.687	0.65	8.646	0.37	2.883	0.42
Heart	13.891	0.46	3.965	0.51	12.237	0.52	3.895	0.56
Liver	81.715	2.68	21.060	2.69	73.677	3.13	17.503	2.52
Spleen	3.847	0.13	1.577	0.20	3.157	0.13	1.514	0.21
Pancreas	6.018	0.20	2.224	0.28	5.007	0.21	1.844	0.27
Right kidney	7.368	0.24	2.265	0.29	7.097	0.30	1.603	0.23
Left kidney	7.392	0.24	2.320	0.30	7.198	0.31	1.716	0.24
Pituitary	0.069	0.0023	0.0394	0.005	0.104	0.0044	0.095	0.014
Thyroid	0.472	0.016	0.190	0.025	0.510	0.023	0.291	0.041
Thymus	3.31	0.11	2.540	0.32	1.379	0.059	0.953	0.13
Right adrenal	0.406	0.013	0.488	0.006	0.487	0.021	0.180	0.0025
Left adrenal	0.486	0.016	0.231	0.029	0.571	0.024	0.198	0.028
Both adrenals	0.893	0.029	0.262	0.033	1.057	0.045	0.365	0.051
Right testicle	0.987	0.032	1.772	0.23	0.515	0.022	0.477	0.069
Left testicle	0.871	0.029	1.392	0.18	0.533	0.023	0.491	0.072

[1] σ, standard deviation.
[2] P.E.M., probable error of the mean.

stance the latter were seen as 2 to 3 mm. whitish bodies lying on the surface of the thyroid.

The thymus was quite the most variable organ found. Size, shape and texture all altered with the condition of the animal. It lies on the ventral surface of the mediastinum, extending from the sternal notch a variable distance toward the diaphragm.

The largest glands extended in two lobes to either side of the pericardium and reached and were attached to the diaphragm. The smallest were non-existent in spite of thorough search.

The thymus of the young and healthy monkeys, either rhesus, anthropoid or other species, was relatively large and consisted of apparently active glandular tissue. Older animals, if still healthy and in good nutritional state showed little decrease in size or weight of this organ, but the grayish glandular tissue was replaced in part or in entirety by yellow fat. No attempt was made to separate these two types of tissue which were throughly intermingled in the structure of the organ.

If, however, the animal belonged to the 'unhealthy' group, showing little subcutaneous fat, and having lost weight, the entire structure whether glandular or fatty tissue was much smaller than in the 'healthy' and well nourished animals.

The pancreas also varied more in weight than many organs, but it decreased in weight with loss of body weight. Its composition seemed uniform grossly in every instance. In these primates it is noteworthy that the head of this organ is particularly difficult to detach from the adjacent structures such as the duodenum and large bowel.

The adrenals are relatively large at birth but after a body weight of about 2,000 gm. is reached they attain a weight which increases proportionally with body weight. The left adrenal is nearly always heavier than the right. The shape alters with pressure from fat and from other abdominal organs.

The reproductive organs were never remarkable in any way except for one undescended testicle in an immature male. The figures for increase in size with body weight can be seen in the tables. Left and right testicles and ovaries usually weighed about the same. The testicles were dissected from the epididymis before weighing.

Of the other organs there is little to be said.

The heart was cut from the large vessels just above the auricles and weighed intact. No disease was ever found in this series of relatively young animals.

The lungs were the organs most frequently showing pathological conditions (4); pneumonia, tuberculosis, or encysted foreign bodies being most common. Those animals which had remained long in the laboratory showed marked anthrocosis. The left lung usually weighed less than the right. Weight of each was altered by congestion, as under anesthetic or when formalin was injected.

Liver and kidney weights varied relatively little except to increase with body weight. Weights of these organs in the 'unhealthy' animals was slightly greater than in the 'healthy.'

The spleen was variable in size, shape and weight. It decreased in weight with loss of body weight.

The gastrointestinal tract was removed, weighed and examined in all cases. Most of the observations are presented in the paper on disease (4), where they are more pertinent, but, in spite of a wide variation in the state of contractility of the tract, its contents and the condition of the mucosa, there was an average condition in the majority of normal animals. In this state, the stomach might be in any condition of dilatation or contractility, depending on the time and amount of food recently eaten. In nearly all cases, however, the small intestine was filled moderately with thin, bile-stained fluid chyle. The large intestine always contained fecal material in its upper two-thirds, while the descending colon, the sigmoid and rectum might be either full or empty. The usual condition of the gut was semi-contracted.

DISCUSSION

Analysis of data. With the advice and guidance of Mr. Frank K. Shuttleworth, these data were analyzed and compared for sex, weight and health. The statistics for

the group of 103 immature animals are reproduced here (table 6). The number of infant and mature animals was too few for such complete analysis.

These statistics include mean organ weights, ratios of mean organ to mean autopsy weights, standard deviations,[2] and probable errors of means separately for the two sexes and for 'healthy' and 'unhealthy' animals. Probable errors of the differences between means have also been computed routinely, but it does not seem necessary to report these. Instead, the discussion which follows notes that certain differences are statistically significant (3 or more times as large as their probable errors) while others are not significant (less than 3 times their probable errors[3]).

Differences due to sex. Comparing the weights of organs in the two 'healthy' groups of 32 males and 34 females, one finds the mean body weight of the immature group of females is 2,923 gm. and that of the immature males is 3,039 gm.; the difference is not significant. The mature males, however, may reach a weight of 8 to 9 kg., whereas the females seldom reach more than 6 to 7 kg. (These higher weights have been found in other animals dying in this laboratory, although in the present series there are none of either sex in the highest weight groups.) There is no weight difference of significance between males and females of the immature group of any organ except the brain. The mean weight of this latter organ for males is 85.7 gm. and 76.5 for the females. In the mature group of 2 males and 3 females average body weight of males is 6,600, and of females 4,833. Average brain weight, males, is 98.1 and females, 86.1 gm. Because of the small number of cases this difference is not statistically significant, but it is in the same direction as that for the immature animals. A similar trend appeared repeatedly in the figures of other investigators for all types of subhuman primates (11, 12).

Differences due to weight (age). The relative rate of growth of organs is shown in tables 4 and 5, in which weight of organs is compared with body weight. It appears that the larger organs, liver, spleen, pancreas, lungs, heart, and thyroid, grow gradually as body weight increases. In contrast, the brain weight increases rapidly with increasing body weight in the smaller animals below 2,500 to 3,000 gm.; but above that body weight, the brain weight increases much more slowly, that of the males slightly more than that of females.

The smaller endocrines increase relatively little in weight with body growth. The gain is gradual throughout the entire growth period.

The thymus glands of the 'healthy' group showed a gradual increase in weight with increase in body weight. But, as has been mentioned previously, it was impossible to separate glandular tissue from fatty deposits within the structure of the gland.

Differences due to condition of health. In both males and females the most striking difference between 'healthy' and 'unhealthy' animals was, of course, in the total body weight, since loss of weight was one criterion for placing individuals in the 'unhealthy' group.

The next most striking difference was in thymus weight. According to Boyd (15) the thymus of the normal human individual increases to puberty and then slowly decreases to senility. She states that after puberty the parenchyma decreases sufficiently to lower the total weight of thymus even though connective tissue and fat are increased. Furthermore, Boyd states that at all ages variability is great, that involution sets in when illness lasts more than 24 hours regardless of the cause of death; and that

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[2] Standard deviation $= \alpha = \sqrt{\frac{\Sigma X^2}{N} - M^2}$ where X is actual weight of every organ, N is number of animals, and M is mean weight of group.

[3] Probable error mean $= \frac{\alpha}{\sqrt{N}} \times 0.6745$.

the reaction of the thymus to disease is like that of the subcutaneous fat and lymphoid tissue.

Although this series is too small to be compared with the 1,800 cases of Boyd, all data agree with her statements. For, both in the group of *Macaca mulatta* and that of the chimpanzees (1), glandular tissue was always greatest in young and healthy animals. It was always least in those which had lost a large amount of weight regardless of the cause and also of the age of the animal. The heaviest thymus weights appeared in the animals with body weights between 3,000 and 4,000 gm. Eleven gm. was the heaviest weight and the lightest were practically nothing, as, in 2 of the 'healthy' group and 8 of the 'unhealthy' it was not found at all. In the heavier animals, however, the glandular tissue was in many cases entirely or partially replaced by fat.

In these subhuman primates indications are, therefore, that the changes which occur in the thymus gland are coincident with growth and with the condition of health, as shown by Boyd for human beings. Large and glandular masses occur in young and healthy animals. If health is maintained through growth, glandular tissue is replaced by fat and there is little loss of total weight to the beginning of maturity at body weights of about 4,500 gm. or more. Any disease or other process producing loss of subcutaneous fat results in marked reduction of the weight of both glandular and fatty tissue in the thymus. Correlation of weight of thymus to lymphoid proliferation did not show simultaneous changes, probably largely because general enlargement of lymphoid tissue was difficult to determine and rarely seen. Local proliferation in or about the gastrointestinal tract or mediastinum showed no consistent changes related to those of thymus.

Calculation of probable error difference between the groups of males, females, 'healthy' and 'unhealthy' shows significant differences for both sexes due to health in body and thymus weights only. In the females there are also significant differences between weights of heart, liver, spleen and pancreas.

Comparison of the ratios of mean organ weights to mean body weights in 'healthy' and 'unhealthy' groups brings out suggestive differences. In the 'unhealthy' group, ratios were always calculated to autopsy weight and not to maximum weight for two reasons: first, because the separation between the two groups was an arbitrary one which was conditioned primarily by the need to establish standards for a group which could be considered healthy in every respect; and second, because the maximum weights were not always comparable as, during life, animals were weighed at irregular intervals and by various methods.

In analysing the group changes, therefore 15 animals in the immature 'unhealthy' group were selected which had lost large amounts of weight but which had no severe infectious process at the time of death. Ratios of mean organ weights to mean maximum weights for this group were much nearer to the ratios of organ to autopsy weight in the 'healthy' group than were the ratios of the same 15 animals figured with organ weight to autopsy weight. This indicates that loss of total body weight is largely due to loss in tissues other than those weighed. Observation at autopsy showed that these changes were chiefly in fat of omentum, mesentery and subcutaneous tissue.

Further comparison of the ratios of the two groups shows individual variation between the different organs (table 6). Spleen and pancreas alone lose weight along with body weight; ovaries and testicles lose relatively less. The remaining endocrines and organs change little as the body loses weight so that the ratios of their individual weights to maximum weights are of the same denomination as the ratios of the healthy group. The thymus weight alone decreases markedly with loss of body weight.

Finally, we would point out that the findings at the autopsies of these 118 *Macaca mulatta* presented in this paper are corroborated and substantiated by the observa-

tions made on anthropoids and on monkeys of other species, although the data on the latter two groups are insufficient for as complete analysis as those of the *Macaca mulatta*.

SUMMARY

Routine consecutive autopsies have been made on 216 *Macaca mulatta*. The effect of sex, weight and health on the weights of brain, endocrines and visceral organs of 118 of these are presented and analysed, with particular attention to a group of 103 immature animals weighing between 1,000 and 4,500 gm.

The weight of the brain alone is influenced by the sex of the animal. The mean brain weight of the males in this group being 85.7, and that of the females 76.5 gm.

Increasing body weight with age is accompanied by a concomitant increase in absolute organ weight; the relative increase is different for the different organs, the small endocrines increasing less than the larger structures. The glandular tissue is less, the fatty tissue of the thymus more in the heavier (older) animals.

Poor health, as indicated by loss of weight in a group of 52 animals without serious gross disease, affects various organs in different ways. The chief loss of weight occurs in structures other than the endocrines, or visceral organs, chiefly in the fatty tissues.

Loss of body weight is accompanied by marked loss of glandular and fatty tissue from the thymus; there is loss of weight in spleen and pancreas, and to a less degree in testicles and ovaries. The weight of the remaining organs is little affected.

The compilation of these data could not have been accomplished without the cooperation and assistance of many people in the Laboratory. Our thanks are due in particular to Professor J. F. Fulton whose concept of the use of subhuman primates for experimental purposes has made this material available; to Mr. Frank K. Shuttleworth for his guidance in compiling the statistical material; and to Dr H. M. Zimmerman for assistance in the examination of pathological material. We are indebted likewise to the staff and technical assistants of the Laboratory for their patience in submitting the material for autopsy over a period of many months.

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FINDINGS AT AUTOPSIES OF SEVENTY ANTHROPOID APES[1]

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THE MATERIAL OFFERED in this paper has been obtained at the autopsies of 70 anthropoid apes during the last ten years.[2] There are many gaps in the data, due to the fact that during the first 8 years the dead animals were often merely by-products of physiological experiments. However, during this earlier period there were consistent careful observations on the central nervous system and, also in many instances, valid organ weights. During the last 2 years systematic autopsies and records have been made by the authors. Since the findings at autopsy of anthropoids appear rarely in the literature and since these animals are probably typical of those obtained by laboratories elsewhere, the weight of organs and the pathological findings are here reported. A more detailed account of similar data on weight of organs and on disease will be found in papers dealing with this material in a larger series of autopsies of monkeys performed in the same laboratory during the past two years (4).[3] Literature concerning previous material on the subject is fully discussed in the accompanying paper on *Macaca mulatta*.

MATERIAL AND METHODS

These anthropoids were 58 chimpanzees, 4 ourang utans, 3 gorillas and 5 gibbons, which had come to the Laboratory for various purposes. 11 (7 chimpanzees, 2 gibbons and 2 gorillas) were dead on arrival (labelled "carcass" in the tables below) and had been sent to us for autopsy examination. Of the remaining 59, a few were acquired in poor health from zoos or dealers for use in acute experiments. The rest were in average health on arrival and were kept on standard diet and uniform conditions in the Laboratory for a period of weeks or months. They were used during that period for experiments in neurophysiology which very often entailed operations on the central nervous system.

The animals were autopsied within a few hours after death except those sent to us dead which had been frozen in dry ice. Those dying in the Laboratory were usually injected with 10% formalin at the time of death. A large series of injected and non-injected *Macaca mulatta* has shown that injection without increase in pressure alters little if at all the weight of any tissue except the lungs.

From the records previous to death it was found that there were 24 chimpanzees, 1 gorilla, 1 ourang utan and 1 gibbon which could be considered normal and the organ weights of which can be compared with less normal animals. Such 'normal' animals were those showing no disease at autopsy and which had not lost more than 10% of body weight as result of an experimental procedure.

Since the series is too short for statistical analysis, the data on all organ weights are offered

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[2] In the Laboratory of Physiology of the Yale School of Medicine.
[3] All references given here are to be found in bibliography of accompanying paper on *Macaca mulatta*.

TABLE 1. BRAIN AND BODY WEIGHTS AT AUTOPSY OF ALL ANTHROPOIDS OF KNOWN AGE

Species	No.	Sex	Age	Weight	
				Autopsy	Brain
				gm.	gm.
Chimpanzee	84	F	3 mo.	2,900	275
	92	F	4 mo.	3,650	248
	73	F	5 mo.	2,100	215
	67	F	12 mo.	3,700	267
	43	F	18–20 mo.	5,500	340
	14	F	mature	17,000	356
	22	F	mature	17,600	388
	13	F	13–14 yr.	33,000	370
Orang utan	4	M	16 mo.	4,500	325
	3	M	2 yr.	6,700	353
	2	F	mature	25,200	340
	1	F	mature	30,000	315
Gorilla	3	F	mature	65,000	431
Gibbon	3	M	mature	5,700	105

together with the ratio of organ weight to autopsy weight. Both autopsy weight and maximum weight are given whenever possible. In the event that no autopsy weight was obtained, the ratios are to maximum weight, as indicated on the tables. All weights are in grams.

The absolute or approximate age of the animals was known in a few instances. These are listed in table 1; in the other animals age or degree of maturity could only be estimated.

The brain weights are of brains after spinal cord has been severed just below the pyramidal decussation. All other organs were weighed intact except in the following instances: parathyroids were not removed from thyroids; in thymus, no attempt was made to separate fatty tissue from glandular; the testicles were weighed without tubules; and the kidneys, after removal of the capsule. The intestinal tract was in many instances opened throughout and carefully examined for mucosal ulcerations. Whenever possible autopsy findings were correlated with observations made during life.

Chimpanzees (Pan)

Of 58 chimpanzees, there were 38 females and 20 males. The greater number were the usual 'immature' animals brought from Africa. There were 4 infants in the first year of life and 3 mature females (table 1). No mature males were examined although numbers 9, 10, 15, 38 and 77 were all approaching sexual maturity at the time of death.

In table 2 is given the cause of death of the 58 animals and in table 3, the pathological findings at autopsy. The incidence of disease is summarized in table 3. In this

TABLE 2. CAUSE OF DEATH OF 58 CHIMPANZEES

Killed for experiment	17
Died as result of experimental procedure	20
Pneumonia	9[1]
Diarrhea	7
Tuberculosis	3
Vincent's infection	1
Peritonitis, postpartum	1[2]
Total	58

[1] Seven of these received dead for autopsy
[2] Received dead for autopsy.

TABLE 3. DISEASE AT AUTOPSY OF 58 CHIMPANZEES

Animal No.		Animal No.	
1	Tuberculosis	38	None
2	Tuberculosis	39	Tuberculosis
4	None	40	Ulcerative colitis
5	Tuberculosis	43	Tuberculosis
6	Tuberculosis	49	None
8	Pneumonia (postoperative)	50	Tuberculosis
9	None	53	None
10	Colitis	54	Colitis
11	Cord transection	55	Vincent's angina
13	None	56	None
14	Colitis	57	Colitis
15	Hemorrhagic colitis	59	Colitis
16	None	63	None
17	None	64	None
18	None	67	Pneumonia
19	Meningitis (postoperative)	68	Pneumonia (carcass)
20	Cysticercus infestation	69	Pneumonia (carcass)
21	None	70	Pneumonia (carcass)
22	Postpartum infection (carcass)	71	Pneumonia (carcass)
25	None	72	Pneumonia (carcass)
26	None	73	Pneumonia (carcass)
27	None	76	None
29	Pneumonia (postoperative)	77	Meningitis (postoperative)
30	None	80	Tuberculosis
31	None	81	None
33	None	84	None
34	Meningitis (postoperative)	87	Tuberculosis
36	None	92	Colitis
37	None	94	Pneumonia

Summary

	Number
Pneumonia	11[1]
Colitis—acute	11
Tuberculosis	7
Meningitis (postoperative)	5
Ulcers of stomach	4
Ulcers of colon	2
Intestinal parasites: Tape worm	1
Round worm	1
Cysticercus	1
Postpartum peritonitis	1[2]
Vincent's angina	1

[1] Seven of these were dead on arrival.
[2] Dead on arrival.

species brain, endocrine and organ weights have been obtained in a considerable series (tables 4–7).

Gorillas (Gorilla gorilla)

There are weights of three gorilla brains (table 8) removed at autopsy. Two of these animals were 'carcasses' dead on arrival and general autopsy was not made. The third, a mature female, was killed in the laboratory and careful examination of all organs was recorded. This animal had been under observation in a zoo for 6 years and during the last 2 had developed paralysis of the lower extremities. These were markedly atrophic although the rest of the animal was normally developed and in excellent

TABLE 4. BRAIN AND BODY WEIGHTS AT AUTOPSY OF 58 CHIMPANZEES

No.	Sex	Body wt.		Brain	Ratio Brain : Autopsy wt.	Cause of death
		Maximum	Autopsy			
		gm.	gm.	gm.		
73	F		2,100	215	10.20	(Carcass)
84[1]	M	2,940	2,900	275	9.82	Post-operation
92[1]	F	4,100[2]	3,650	248	6.46	Diarrhea
67	F	4,000[2]	3,700	267	7.23	Pneumonia
70	M		4,200	280	6.66	(Carcass)
43	F	6,600	5,500	340	6.18	Tuberculosis
57	M	7,300	5,650	322	5.70	Diarrhea
54	F	8,600[2]	6,200	337	5.43	Diarrhea
55	F	7,650		350	4.57	Operation
29	F	8,000[2]	7,700	282	3.67	Pneumonia
69	M		8,400	358	4.26	(Carcass) pneumonia
71	F		9,900	335	3.38	(Carcass) pneumonia
94	F		11,200	340	3.03	Pneumonia
68	F		11,500[2]	325	2.82	(Carcass)
37[1]	F		11,700	320	2.73	Killed
36[1]	F		11,900[2]	335	2.75	Killed
2	F	13,000	12,400	290	2.33	Killed
53[1]	F	12,500	12,500[2]	312	2.49	
50	F	14,500	12,500[2]	345	2.76	Tuberculosis
87	M	13,100		362	2.76	Tuberculosis (emaciated)
63	M	14,500	13,800	373	2.69	Operation
8[1]	M	14,600	14,000	342	2.44	Killed (pneumonia)
4[1]	F	14,000	14,000	324	2.17	Operation
77	M	17,300	14,500	403	2.77	Meningitis
59	F	15,200[2]	15,200	385	2.53	Diarrhea (thin)
27[1]	F	15,250	15,250	350	2.29	Operation
11	M	19,000	16,500	355	2.15	Cord transection
14	F	35,000	17,000	356	1.03	(Emaciated)
18[1]	F		17,300[2]	334	1.98	Operation
56[1]	M	19,800	17,300	347	2.00	Post-operation
22	F		17,600[2]	388	2.20	(Carcass) postpartum
17[1]	M	19,100	18,800	343	1.82	Operation
1	M	24,700	19,400	335	1.72	Killed (tuberculosis)
81[1]	F	20,400[2]	20,400	355	1.74	Operation
64[1]	F	20,900	20,900	330	1.57	Operation
25[1]	M	21,550		349	1.71	Killed
33[1]	F	21,700	21,700	364	1.67	Operation
76[1]	F	23,400	23,400	338	1.44	Operation
15	M	35,000	24,000	345	1.41	Post-operation (diarrhea)
40	F	24,600[2]	24,600	321	1.30	Post-operation (diarrhea)
16[1]	F	25,400[2]	25,000	361	1.41	Killed
31[1]	M	26,000		368	1.41	Operation
72	F		26,200	375	1.43	(Carcass) pneumonia
38[1]	M	27,400	27,400	365	1.33	Killed
10	M	31,300	28,800	371	1.28	Diarrhea
21[1]	F	38,300[2]	30,100	346	1.15	Killed
20	F	34,000[2]	34,000	374	1.10	Killed
6	F	36,500		319	0.87	Killed
13[1]	F	36,700[2]		370	1.0	Killed
9[1]	M	38,300	37,000	379	1.02	Operation

[1] Normal animals.
[2] Injected at autopsy (cf. Table 5).

nutritional state and health. The body weight of 65 kg. is, however, undoubtedly low for a mature female and ratio of organ to body weight consequently high (table 9).

Orang Utan (Pongo)

There is little to be noted concerning the orang utans beyond facts given in the tables 8 and 9. Numbers I and II were mature females, the mothers of numbers III and IV

TABLE 5. CHIMPANZEE ENDOCRINE WEIGHTS

No.	Body wt.	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Gonads	
						Right	Left	Right	Left
				Female					
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
92[1]	3,650[2]		1.72 (0.0471)[6]	13.21 (0.362)[6]	5.4 (0.147)[6]	1.78 (0.0487)[6]	2.43 (0.0665)[6]	0.79 (0.0216)[6]	0.79 (0.0216)[6]
67	4,060[2]	0.0975 (0.0024)[6]	1.77 (0.0436)	6.27 (0.154)	11.0 (0.27)	1.85 (0.0455)	2.55 (0.0628)	0.45 (0.011)	0.40 (0.00985)
54	6,200	0.1212 (0.00195)	1.069 (0.0172)	1.839 (0.0296)	18.629 (0.3)	1.624 (0.0262)	1.826 (0.0294)	0.307 (0.00495)	
29	7,700[3]				14.0 (0.181)	4.0 (0.0519)	4.0 (0.0519)		
30	9,500[3]				25.0 (0.263)	5.0 (0.0526)	4.8 (0.0505)		
71	9,900			4.3 (0.0434)					
34	10,400[4]		3.0 (0.0288)			6.0 (0.0577)	5.0 (0.048)		
94	11,200	0.16 (0.00142)	0.98 (0.00875)	3.38 (0.0301)	30.74 (0.274)	1.77 (0.0158)	2.32 (0.0207)	0.44 (0.00393)	0.43 (0.00384)
68	11,500[2]	0.1713 (0.00148)	1.251 (0.0108)	1.0 (0.0087)	11.631 (0.101)	2.307 (0.02)	2.253 (0.0195)	0.156 (0.00135)	
36	11,900[3]					5.0 (0.0446)	5.0 (0.0446)		
53[1]	12,500[2]			34.2 (0.273)		1.2 (0.0096)	1.75 (0.014)		
50	12,500[2]	0.278 (0.00222)		5.2 (0.0416)		3.44 (0.0275)	3.96 (0.0316)		
59	15,200[2]	0.2964 (0.00195)	2.101 (0.0138)	4.115 (0.027)	17.82 (0.117)	2.705 (0.0177)	3.72 (0.0244)	0.9675 (0.00636)	
27[1]	15,250		2.36 (0.0154)			4.3 (0.0282)	4.7 (0.0308)		
18[1]	16,450[2]		5.0 (0.0304)	50.0 (0.304)	40.0 (0.243)	3.0 (0.0182)	3.5 (0.0212)	1.0575 (0.00642)	0.8675 (0.00527)
22	17,630[2]	0.602 (0.00341)	6.35 (0.036)		25.0 (0.141)	4.1 (0.0232)	4.8 (0.0272)		
81[1]	20,400[2]	0.31 (0.00152)	2.2 (0.0107)	28.5 (0.139)	44.0 (0.215)	2.23 (0.0109)	2.22 (0.0108)	0.85 (0.00416)	0.83 (0.00406)
64[1]	20,900	0.432 (0.00206)	4.375 (0.0209)	22.091 (0.105)	22.613 (0.108)	2.216 (0.0106)	1.742 (0.00833)	2.5496 (0.0122)	1.9623 (0.0094)
76[1]	23,400	0.5 (0.00213)	2.93 (0.0125)	23.05 (0.0985)	57.08 (0.243)	2.9 (0.0124)	3.84 (0.0164)	1.54 (0.00658)	1.20 (0.00512)
40	24,600[2]	0.27 (0.00109)	4.79 (0.0194)	3.71 (0.015)	29.53 (0.12)	4.36 (0.0177)	6.2 (0.0252)	2.56 (0.0104)	2.3 (0.00935)
16[1]	25,000[4]				43.0 (0.172)	1.8 (0.072)	2.0 (0.08)		
72	26,200	0.376 (0.00143)	1.613 (0.00615)	12.316 (0.047)		4.205 (0.016)	5.448 (0.0207)	0.72 (0.00274)	0.914 (0.00348)
21[1]	30,100	0.4273 (0.00142)	3.859 (0.0128)	6.705 (0.0222)	26.0 (0.0863)	2.658 (0.00833)	3.273 (0.0108)	3.88 (0.0128)	
20	34,000[2]	0.54 (0.00158)	4.793 (0.0141)	20.813 (0.0612)	33.0 (0.097)	2.39 (0.00703)	2.776 (0.00816)	1.513 (0.00445)	0.9858 (0.00289)
13[1]	36,700[5]	0.396 (0.00107)						2.302 (0.00627)	1.772 (0.00482)
				Male					
84[1]	2,900[2]	0.15 (0.00517)	0.41 (0.0141)	4.31 (0.148)	3.24 (0.111)	0.57 (0.0196)	0.64 (0.022)	0.58 (0.02)	0.64 (0.022)
70	4,200			3.15 (0.075)		2.25 (0.0535)	1.86 (0.0443)		

TABLE 5—*Continued*

No.	Body wt.	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Gonads	
						Right	Left	Right	Left
					Male				
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
57	5,650	0.078 (0.00138)	2.155 (0.0381)	1.23 (0.0217)	10.144 (0.179)	1.7925 (0.0317)	2.268 (0.0401)	3.7025 (0.0655)	
69	8,400[2]	0.107 (0.00127)	1.546 (0.0184)	1.133 (0.0134)	6.391 (0.076)	2.144 (0.0255)	2.502 (0.0297)	2.817 (0.0335)	
63[1]	13,850	0.28 (0.00202)	2.436 (0.0175)	14.2 (0.102)	21.45 (0.154)	2.1 (0.0151)	2.1 (0.0151)	0.89 (0.00642)	1.11 (0.00801)
49	14,200[2]	0.1579 (0.00111)	2.425 (0.017)	10.146 (0.0714	15.807 (0.111)	1.919 (0.0135)	1.963 (0.0138)	5.655 (0.0398)	
77	14,500[2]	1.5 (0.0142)	1.83 (0.0126)	4.3 (0.0296	25.0 (0.172)	3.4 (0.0234)	3.5 (0.0241)	34.17 (0.235)	34.17 (0.235)
56[1]	17,300		2.79 (0.0161)	14.62 (0.0845)	22.94 (0.132)	2.72 (0.0157)	3.25 (0.0187)	6.1 (0.0352)	7.15 (0.0413)
25[1]	21,550[3]					5.0 (0.0232)	5.0 (0.0232)		
31[1]	26,000	0.36 (0.00138)	1.9 (0.0073)	28.652 (0.1102)		2.0 (0.00769)	2.278 (0.00876)	14.192 (0.0545)	
9[1]	37,000[2]	0.39 (0.00105)	4.8 (0.0129)			4.0 (0.0108)	5.0 (0.0135)		

[1] Normal.
[2] Injected with formalin at autopsy.
[3] Injected with alcohol at autopsy.
[4] Injected with Müller's solution at autopsy.
[5] Injected with chloral hydrate at autopsy.
[6] Ratio of endocrine weight to body weight in parentheses.

respectively. All were in fair health at the time of death, but only number II could be considered 'normal.'

Gibbons (Hylobates)

Of the gibbons, organ weights were obtained in two instances,—number V, a 'carcass,' dead of lobar pneumonia, and number III, a 'normal' mature male killed in the laboratory (tables 8 and 9). Numbers I, III, and V were gray animals probably of the species *Hylobates lar*, number II was black, *Hylobates leucogenys* and number IV a mature male *Hylobates symphalangus syndactilus*, the brain of which was sent to us from Java.

DISCUSSION

Weights

Brain. All the data concerning brain weights can be obtained from tables 1, 4 and 8.

During the first year of life, or until the chimpanzee weighs between 6 and 10 kg., the brain grows rapidly to a weight of between 320 and 350 gm. It increases little thereafter but the ratio of brain weight to body weight steadily decreases until a body weight of 25 kg. or more is attained. No deductions can be made as to the effect of age on the other anthropoids, but the same early development of brain as compared to body is indicated in that data for orangutans. It appears as well in *Macaca mulatta* and other 'lower' primate forms.

TABLE 6. CHIMPANZEE ORGAN WEIGHTS (FEMALE)

No.	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Right	Left				Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
92[1]	3,650[2]	22.25	19.15	29.4	155.0	9.35	18.55	19.24
		(0.609)[5]	(0.524)[5]	(0.805)[5]	(4.24)[5]	(0.256)[5]	(0.508)[5]	(0.527)[5]
67	4,060[2]			50.0	191.0	30.0	63.0	58.0
				(1.23)	(4.7)	(0.738)	(1.55)	(1.42)
54	6,200	36.0	36.0	38.0	218.0	35.0	43.0	48.0
		(0.58)	(0.58)	(0.612)	(3.51)	(0.564)	(0.693)	(0.774)
29	7,700[3]				355.0	19.0	21.0	21.0
					(4.61)	(0.246)	(0.272)	(0.272)
30	9,500	45.0	45.0	55.0	400.0	55.0	50.0	55.0
		(0.473)	(0.473)	(0.578)	(4.21)	(0.578)	(0.526)	(0.578)
34	10,400[4]	30.0	30.0	48.0	340.0	37.0	35.0	35.0
		(0.288)	(0.288)	(0.461)	(3.27)	(0.355)	(0.336)	(0.336)
94	11,200			55.48	375.0	37.12	31.33	34.7
				(0.495)	(3.34)	(0.331)	(0.279)	(0.309)
37	11,700[4]	70.0	55.0	65.0	490.0	60.0	50.0	48.0
		(0.598)	(0.47)	(0.555)	(4.18)	(0.512)	(0.427)	(0.41)
36[1]	11,900[3]	100.00	90.00	50.00	330.0	40.00	30.00	30.00
		(0.84)	(0.756)	(0.42)	(2.77)	(0.336)	(0.252)	(0.252)
50	12,500[2]			76.00	438.0	40.00	45.00	52.00
				(0.608)	(3.504)	(0.32)	(0.36)	(0.416)
59	15,200[2]	120.0	114.0	80.0	595.0	47.0	66.0	68.0
		(0.789)	(0.75)	(0.526)	(3.91)	(0.309)	(0.434)	(0.447)
27[1]	15,250			58.00		53.0	32.0	24.0
				(0.38)		(0.347)	(0.209)	(0.157)
18[1]	16,450[2]	115.0	105.0	90.0	565.0	70.0	65.0	72.0
		(0.699)	(0.638)	(0.547)	(3.43)	(0.425)	(0.395)	(0.437)
22	17,630[2]			91.00	536.0	37.0	78.0	
				(0.516)	(3.04)	(0.209)	(0.442)	
81[1]	20,400[2]			119.0	375.0	55.0	61.0	60.0
				(0.583)	(1.83)	(0.269)	(0.299)	(0.294)
64[1]	20,900			84.00		105.00		
				(0.401)		(0.502)		
39	23,000[2]	150.0	125.0	135.0	1080.0	85.0	75.0	83.0
		(0.652)	(0.543)	(0.587)	(4.69)	(0.369)	(0.326)	(0.36)
76[1]	23,400	128.0	95.0	102.0	700.0	57.0	60.6	43.62
		(0.547)	(0.405)	(0.435)	(2.99)	(0.243)	(0.258)	(0.186)
40	24,600[2]	101.00	100.00	125.00	900.0	46.28	87.23	89.81
		(0.41)	(0.406)	(0.508)	(3.65)	(0.188)	(0.354)	(0.365)
16[1]	25,000[4]			119.0	698.0	65.0	76.0	75.0
				(0.476)	(2.79)	(0.26)	(0.304)	(0.3)
19	27,000	225.0	300.0	150.0	1150.0	125.0	125.0	150.0
		(0.833)	(1.11)	(0.555)	(4.25)	(0.463)	(0.463)	(0.555)
21[1]	30,100[2]	100.0	186.0	120.0	743.0	95.0		70.0
		(0.332)	(0.617)	(0.398)	(2.46)	(0.315)		(0.232)
20	34,000[2]			196.0				
				(0.576)				
14	35,000[2]	78.00	95.0	90.0	567.0			86.0
		(0.222)	(0.271)	(0.257)	(1.62)			(0.245)

[1] Normal.
[2] Injected with formalin at autopsy.
[3] Injected with alcohol at autopsy.
[4] Injected with Müller's solution at autopsy.
[5] Ratio of organ weight to body weight in parentheses.

Sex has little effect on brain weight if the ratios of brain to body weight are compared, but brain and body weight of adult animals are both less in the female than in the male chimpanzee.

The weight of organs in disease must be influenced by the changes in fluid balance in the body. Hence the chimpanzee group has been divided into 'normal' and 'ab-

TABLE 7. CHIMPANZEE ORGAN WEIGHTS (MALE)

No.	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Right	Left				Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
84[1]	2,900[2]			14.71 (0.507)[4]	82.16 (2.83)[4]	18.00 (0.62)[4]	8.94 (0.308)[4]	9.12 (0.314)[4]
57	5,650	49.0 (0.867)[4]	46.0 (0.814)[4]	37.00 (0.655)	248.00 (4.38)	28.00 (0.495)	38.00 (0.672)	34.00 (0.601)
63[1]	13,850	48.0 (0.346)	45.0 (0.324)	100.0 (0.722)		110.0 (0.794)	40.33 (0.291)	42.19 (0.304)
49	14,200[2]	55.0 (0.387)	50.0 (0.352)	75.00 (0.528)	508.00 (3.57)	58.00 (0.408)	41.00 (0.288)	41.00 (0.288)
77	14,500[2]	71.5 (0.493)	58.0 (0.4)	81.50 (0.562)	496.00 (3.42)	43.50 (0.3)	50.33 (0.347)	48.84 (0.336)
56[1]	17,300	122.0 (0.705)	100.0 (0.578)	102.00 (0.589)	760.00 (4.39)	43.09 (0.249)	51.84 (0.299)	51.32 (0.296)
1	19,400[2]			153.00 (0.788)	670.00 (3.45)	190.00 (0.979)		
25	21,550[3]	92.0 (0.426)	80.0 (0.371)	125.00 (0.58)	615.00 (2.85)	90.00 (0.417)	68.00 (0.315)	83.00 (0.385)
15	24,400	95.0 (0.389)	95.0 (0.389)	155.00 (0.635)	687.00 (2.81)	70.00 (0.286)	80.00 (0.327)	75.00 (0.307)
38[1]	27,400[2]			136.00 (0.496)	600.00 (2.18)	96.00 (0.35)		

[1] Normal.
[2] Injected with formol at autopsy.
[3] Injected with alcohol at autopsy.
[4] Ratio of organ weight to body weight in parentheses.

normal' animals, and autopsy diagnosis noted (table 3). In the 'abnormal' group, general dehydration must account for some low weights, and local intracranial changes for some increases. However, in general brain weights seem to correlate better with autopsy weights than with maximum weights, indicating that as body weight varies there is adjustment in the water content of the brain. The changes in brain weight under conditions of altered body weight are not as extreme as they are in some of the more distensible organs such as liver, spleen or kidneys.

Endocrines. (Tables 5, 9). The pituitary weights and ratios are fairly consistent with two exceptions. One of these (No. 77) died with meningitis, and it was noted

TABLE 8. BRAIN AND BODY WEIGHTS OF 3 GORILLAS, 4 ORANG UTANS AND 5 GIBBONS

No.	Age	Sex	Body wt., maximum	Body wt., autopsy	Brain	Brain wt. Body wt.	Cause of death
Gorilla I	Immature	F			398		(Carcass)
II	Young	M		4,650	378	8.12	Pneumonia (carcass)
III[1]	Mature	F		65,000	431	.663	Experiment
Orang Utan I	Mature	F		30,000	315	1.05	Meningitis
II[1]	Mature	F		25,200	340	1.34	Asphyxia
III	2 yr.	M	6,700	6,700	353	5.26	Influenza
IV	16 mo.	M	4,500	4,500	325	7.22	Influenza
Gibbon I	Immature	M	2,100	2,100	80	3.8	Experiment
II	Immature	F	2,400	1,800	95	5.2	Experiment
III[1]	6–7 yr.	M	6,200	5,700	105	1.84	Experiment
IV	Mature	?			93		Experiment
V	Mature	M		5,500	109	1.98	Pneumonia (carcass)

[1] Injected with alcohol at autopsy.

at autopsy that the pituitary was enlarged and infected. The second animal (No. 22) with a very large pituitary was a mature female which had died postpartum.

The thyroids varied moderately in weight without much relation to age, body weight or sex. Nor could any other reason for the variations be established by correlation with the weights of the other endocrines.

The thymus weight in the chimpanzee, as in other species (4) varies enormously. There is no relation between sex or size, but the adults have smaller glands and ones in which fatty tissue has replaced much of the glandular. There is in this organ a

TABLE 9. ORGAN AND ENDOCRINE WEIGHTS OF 3 GORILLAS, 4 ORANG UTANS AND 5 GIBBONS

Species	Organ weights							
	Body	Lungs		Heart	Liver	Spleen	Kidneys	
		Right	Left				Right	Left
Gorilla III[1]	65,000	303.2 (0.466)[2]	217.7 (0.334)[2]	394.1 (0.606)[2]	1500.0 (2.307)[2]	192.80 (0.296)[2]	133.90 (0.206)[2]	122.00 (0.187)[2]
Gibbon III[1]	5,700	20.0 (0.35)	17.0 (0.298)	38.0 (0.666)	150.0 (2.63)	14.00 (0.245)	15.00 (0.263)	15.00 (0.263)
Gibbon V	5,500			47.65 (0.866)	178.0 (3.23)	10.67 (0.194)	14.02 (0.254)	16.01 (0.291)

Species	Endocrine weights							
	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Testicles	
					Right	Left	Right	Left
Gorilla III[1]	0.95 (0.00146)	10.03 (0.0154)	28.0 (0.043)	109.80 (0.168)	5.96 (0.00916)	7.43 (0.0114)		
Gibbon III[1]	0.085 (0.00149)		Not found					
Gibbon V		1.60 (0.029)	Vestigial	5.98 (0.108)	0.53 (0.00964)	0.68 (0.0123)	2.69 (0.0489)	2.84 (0.0516)

[1] Injected with alcohol at autopsy.
[2] Figures in parentheses are ratios of organ weight to body weight.

marked difference between the 'normal' and 'abnormal' group. Data on the larger series of macaques show even more clearly than is indicated here that, following either loss of weight, long-standing chronic disease, or sudden infection such as pneumonia, the thymus gland is found to be conspicuously small, often in proportion to the severity of the disease. This is true at least for all but the mature animals, but in the latter group atrophy of thymus seems to occur solely as a result of age.

Of the pancreas little can be said. It increases in size with body weight, but there were marked variations in the ratio of this gland to body weight.

The adrenals, relatively large in infancy, gradually increase in size to maturity. There is usually a significant difference between the weight of the left and the right. Usually (all but 3 cases) the left is larger than the right. The shape was always adapted to the space left by the kidney and other organs, and by the fat of mesentery and omentum.

Testicles were weighed in 7 animals, but in 3 of these only the combined weights of both were given. One male (No. 77) weighing only 14.5 kg. at death and 17.3 6 months before, had testicles weighing 34 gm. each, or more than twice those of number 31 which had a body weight of 26 kg. at death. Data are insufficient to indicate

whether or not number 77 was maturing early. It was the same animal which had an unusually heavy pituitary and which died of meningitis. No other endocrines or organs of this animal had comparably high weights.

The ovaries were weighed in 15 instances, but in 4 of these only the combined weights were recorded. Insufficient records had been kept as to the maturity of these females to warrant deduction as to ovarian function. Only a few of those marked "mature" in table 1 had been observed to have menstrual bleeding.

Organs. The lungs varied considerably in weight, due, except in instances of disease, to the state of dilatation of the blood vessels and congestion of the lungs at the time of death. The anesthetic must have influenced this and injection with formalin in some cases was thought to increase the weight of the lungs. There seems to be no interpretation of these lung weights which is of any other significance.

The heart weight increased, but the ratio to body weight was about the same with growth. No pathology of heart or of aortic arch was ever noted.

The liver weight increased with body weight. But its weight in normal animals was consistently less than in those with disease, indicating congestion in the latter group.

Spleen weights were very variable, like thymus weights, but, unlike the latter, variation bore no relation to health.

The kidney weights, like those of the liver, increased gradually and the weights of 'normal' group were lower than of those with disease.

Abnormal Findings

The incidence and type of pathological findings in the chimpanzee are recorded in tables 2 and 3. They are here reported in connection with the variations in weight of viscera and brain and should be compared with the abnormal findings in the monkey series.

Pneumonia, present in 11 cases, caused death in 9. Seven of these were 'carcasses' and had lobar pneumonia contracted while the animal was en route from Africa. The remaining 4 were those of bronchopneumonia in debilitated animals following cerebral operation.

Diarrhea is a frequent source of weight loss and was the primary cause of death in 7 of 11 cases. Investigation has shown that infestation with *strongyloides* and *Balantidium coli* are the most frequent cause. Tape worm (*Taenia*) also have been found. Occasionally acute bacterial dysentery has swept the colony.

Tuberculosis appeared in 7 of 58 chimpanzees, or 12% and caused the death of 3. Its form, as in the lower primates, is of a rapidly progressing type appearing in lungs or widespread throughout the organs in miliary form. Incidence in the monkeys has been as high as 30% in this colony.

The ulcers and intestinal parasites appear in anthropoids as in the monkeys, and the other occasional findings are recorded in the tables.

SUMMARY

The autopsy findings of 58 chimpanzees, 3 gorillas, 4 orang utans and 5 gibbons have been reported. Organ weights, brain and endocrine weights are recorded and compared as to sex, age and health. The pathological findings are also given.

WEIGHTS OF BRAINS AND ORGANS OF 132 NEW AND OLD WORLD MONKEYS[1]

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THE MATERIAL here presented has been derived from the autopsy records of 132 monkeys of various New and Old World species, and has been compiled together with similar material on 246 *Macaca mulatta* and 70 anthropoid apes published in conjunction with the present paper.

The literature concerning such data has been discussed in the paper on *Macaca mulatta*. There, also, in the larger series of complete autopsies, evaluation of the statistical data has been made. All autopsy procedure and compilation of figures has been done in the same way in all three groups,—the grouping into anthropoids, *Macaca mulatta* and "other monkeys" was made largely for convenience.

In all papers the animals are those used in the Laboratory of Physiology, Yale School of Medicine, during the past 10 years. All *Macaca mulatta* were examined during the last two years by one or both of the authors as were many of the anthropoids and the miscellaneous group of monkeys reported herewith. The brain, organ

TABLE 1. BRAIN AND BODY WEIGHTS OF 17 SOOTY MANGABEYS (*Cercocebus torquatus atys.*)

No.	Sex	Body wt.		Brain wt.	Ratio Brain/Autopsy	Died
		Maximum	Autopsy			
		gm.	gm.	gm.		
972	M	2,350	1,900	70.79	3.71	Killed, emaciated
1324	F	2,600	2,000	83.58	4.18	Malnutrition
1218	F	2,200	2,200	90.23	4.10	Postop. pneumonia
1219	M	2,300	2,300	98.78	4.29	Killed, normal
1274	M	3,500	2,800	113.74[1]	4.06	Postoperative
1326	F	2,850	2,850	99.52	3.49	Unknown
1065	M	3,100	3,100	101.05	3.37	Killed, normal
1328	F	3,250	3,250	98.0	3.01	Killed, normal
1066	M	3,600	3,600	112.7	3.13	Killed, normal
1151	F	5,200	3,700	126.0	3.40	Postoperative
1325	M	3,850	3,850	110.0	2.86	Pneumonia
1245	F	4,400	4,100	87.46	2.12	Hyperthermia, tuberculosis
901	F	4,800	4,400	89.0	2.02	Killed, normal
904	M	5,950	5,100	108.65	2.13	Killed, normal
1155	F	6,100	6,100	95.0	1.55	Killed, normal
905	M	?	6,200	101.4	1.06	Miliary tuberculosis
D[2]	M	?	8,680	140.0	1.61	Wasted hind extremities (mature)

[1] Probably postoperative edema.
[2] Mature.

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[1] These studies have been successively aided by grants from the Fluid Research Funds, Yale University, the Friedsam Foundation and the John and Mary Markle Foundation.

TABLE 2. SOOTY MANGABEY ENDOCRINE WEIGHTS

No.	Body wt.	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Gonads	
						Right	Left	Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Male									
972	1,900[2]	0.07 (0.00368)[3]	0.59 (0.031)[3]	0.24 (0.0126)[3]	3.05 (0.16)[3]	0.25 (0.0131)[3]	0.31 (0.0163)[3]	0.14 (0.00736)[3]	0.14 (0.00736)[3]
1219[1]	2,300		0.44 (0.0191)	1.96 (0.0852)	3.34 (0.145)	0.34 (0.0147)	0.38 (0.0165)	0.33 (0.0143)	0.34 (0.0147)
1274	2,800	0.14 (0.005)	0.92 (0.0328)	1.72 (0.0614)	3.06 (0.109)	0.48 (0.0171)	0.60 (0.0214)	0.64 (0.0228)	0.68 (0.0242)
1065[1]	3,000[2]	0.04 (0.00133)	0.96 (0.032)	5.13 (0.171)	6.52 (0.217)	0.71 (0.0236)	0.72 (0.024)	0.23 (0.00766)	0.26 (0.00866)
1068	3,200	0.05 (0.00156)	0.67 (0.0209)		3.25 (0.101)	0.44 (0.0137)	0.52 (0.0162)	0.33 (0.0103)	0.30 (0.00937)
1066[1]	3,600[2]	0.07 (0.00194)	0.88 (0.0244)	4.36 (0.121)	6.67 (0.185)	0.86 (0.0238)	1.12 (0.0311)	0.32 (0.00889)	0.27 (0.0075)
1325	3,850	0.07 (0.00181)	1.49 (0.0386)	8.03 (0.208)	4.90 (0.127)	0.44 (0.0114)	0.58 (0.015)	0.33 (0.00857)	0.33 (0.00857)
904[1]	5,100[2]	0.14 (0.00274)	1.55 (0.0304)	4.40 (0.0862)	9.38 (0.183)	0.70 (0.0137)	0.62 (0.0121)	0.95 (0.0186)	0.92 (0.018)
D	8,680[2]	0.12 (0.00138)	0.74 (0.00852)	Not found	10.77 (0.124)	0.48 (0.00553)	0.69 (0.00795)	12.78 (0.147)	12.33 (0.142)
Female									
1324	2,000	0.12 (0.006)	0.67 (0.0335)	Not found	6.17 (0.308)	0.45 (0.0225)	0.53 (0.0265)	0.11 (0.0055)	0.12 (0.006)
1218	2,200[2]	0.16 (0.00727)	0.37 (0.0168)	1.56 (0.0709)	3.25 (0.147)	0.52 (0.0236)	0.56 (0.0254)	0.07 (0.00318)	0.10 (0.00454)
1326	2,850	0.06 (0.0021)	0.43 (0.015)	4.49 (0.157)	2.68 (0.094)	0.32 (0.0112)	0.38 (0.0133)	0.06 (0.0021)	0.05 (0.00175)
1328[1]	3,250[2]	0.06 (0.00184)	1.38 (0.0424)	3.73 (0.114)	5.73 (0.176)	0.36 (0.011)	0.41 (0.0126)	0.11 (0.00338)	0.10 (0.00307)
1151	3,700[2]	0.48 (0.0129)	1.35 (0.0364)	1.18 (0.0318)	4.22 (0.114)	1.01 (0.0273)	1.11 (0.03)	0.45 (0.0121)	0.48 (0.0129)
1245	4,100[2]	1.00 (0.0244)	1.12 (0.0273)	5.84 (0.142)	5.91 (0.144)	0.93 (0.0226)	0.93 (0.0226)	0.75 (0.0183)	0.61 (0.0148)
1154	4,300		0.91 (0.0211)	0.36 (0.00837)	5.80 (0.134)	1.02 (0.0237)	0.96 (0.0223)	0.60 (0.0139)	0.50 (0.0116)
901[1]	4,400[2]	0.17 (0.00386)	2.85 (0.0647)	3.60 (0.0818)	6.65 (0.151)	0.60 (0.0136)	0.68 (0.0154)	0.35 (0.00795)	0.30 (0.00682)
1155[1]	6,100	0.13 (0.00213)	0.192 (0.00314)	Not found	6.00 (0.0983)	1.087 (0.0178)	1.166 (0.0191)	0.212 (0.00347)	0.256 (0.00419)

[1] Normal.
[2] Injected with formalin.
[3] Figures in parentheses are ratios of endocrine to body weights.

and endocrine weights of such are given. Only the brain and body weights are reported, however, from the records made during the 8 previous years. These had accumulated during this period of research on the central nervous system, when complete examination of the other organs were in many cases not made.

DATA

a) Complete autopsies. Complete autopsies were made on 17 sooty mangabeys (*Cercocebus torquatus atys*), 1 brown mangabey (*Cercocebus galeritus chrysogaster*), 1 Java monkey (*Macaca irus*), 1 hybrid (mother, *Macaca mulatta;* father, probably

Macaca irus), and 2 tarsius (*Tarsius spectrum*). The weights of organs are given in tables 1–5.

The series number of each animal is recorded for identification in all tables. Weights are arranged in ascending order of body weights as determined at autopsy. Additional data such as maximum weight and disease are given in the tables of brain weights only. 'Normal' animals noted are those in which no disease was found and which had not lost more than 10% of body weight at the time of autopsy. The ratio of brain, endocrine or organ weight to body weight at autopsy has been calculated, and these figures are those in parentheses in the tables.

TABLE 3. SOOTY MANGABEY ORGAN WEIGHTS

No.	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Right	Left				Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
				Male				
972	1,900[2]	10.73 (0.564)[3]	9.73 (0.512)[3]	9.90 (0.521)[3]	86.04 (4.53)[3]	3.33 (0.175)[3]	8.19 (0.431)[3]	7.66 (0.403)[3]
1219[1]	2,300	6.49 (0.282)	5.20 (0.226)	10.07 (0.437)	64.38 (2.79)	2.79 (0.121)	7.81 (0.339)	7.81 (0.339)
1274	2,800	9.39 (0.335)	7.80 (0.278)	12.84 (0.458)	91.29 (3.26)	3.19 (0.114)	7.38 (0.263)	7.30 (0.26)
1065[1]	3,000[2]	13.47 (0.449)	12.23 (0.407)	17.30 (0.576)	66.44 (2.21)	4.29 (0.143)	8.72 (0.29)	9.67 (0.322)
1068	3,200	7.34 (0.229)	5.98 (0.186)	10.94 (0.342)	97.15 (3.03)	4.05 (0.126)	8.50 (0.265)	8.82 (0.275)
1066[1]	3,600[2]	7.87 (0.218)	4.87 (0.135)	6.28 (0.174)	78.49 (2.18)	4.86 (0.135)	11.06 (0.307)	11.28 (0.313)
1325	3,850	13.92 (0.361)	11.51 (0.299)	16.44 (0.427)	94.20 (2.446)	4.46 (0.115)	8.14 (0.211)	7.93 (0.206)
904[1]	5,100[2]	13.57 (0.266)	11.34 (0.222)	22.35 (0.438)	103.50 (2.03)	10.30 (0.202)	12.31 (0.241)	12.35 (0.242)
D	8,680[2]	39.66 (0.456)	39.56 (0.455)	62.48 (0.719)	160.00 (1.84)	6.76 (0.0779)	22.96 (0.264)	19.56 (0.225)
				Female				
1324	2,000	6.46 (0.323)	5.40 (0.27)	16.68 (0.834)	73.65 (3.682)	2.43 (0.121)	6.34 (0.317)	6.57 (0.328)
1218	2,200[2]	16.00 (0.727)	11.30 (0.513)	10.72 (0.487)	61.16 (2.78)	2.73 (0.124)	6.71 (0.305)	6.35 (0.288)
1326	2,850	8.36 (0.293)	8.09 (0.283)	18.90 (0.663)	85.45 (2.99)	3.78 (0.132)	8.85 (0.31)	8.61 (0.302)
1328[1]	3,250[2]	11.68 (0.359)	9.13 (0.28)	13.73 (4.22)	70.55 (2.17)	4.53 (0.139)	9.46 (0.291)	9.02 (0.277)
1151	3,700[2]	16.45 (0.444)	25.60 (0.692)	18.36 (0.496)	135.00 (3.64)	7.49 (0.202)	11.51 (0.311)	11.21 (0.303)
1245	4,100[2]	47.81 (1.16)	28.32 (0.69)	20.94 (0.51)	125.00 (3.05)	14.76 (0.36)	12.56 (0.306)	11.33 (0.276)
1154	4,300	22.45 (0.522)	17.20 (0.40)	26.65 (0.62)	200.00 (4.65)	7.80 (0.181)	16.30 (0.379)	17.60 (0.409)
901[1]	4,400[2]	10.00 (0.227)	12.60 (0.286)	15.70 (0.356)	115.00 (2.61)	5.10 (0.115)	12.85 (0.292)	13.30 (0.302)
1155[1]	6,100	18.00 (0.295)	20.00 (0.327)	26.00 (0.426)	157.00 (2.57)	9.00 (0.147)	16.00 (0.262)	15.00 (0.245)

[1] 'Normal.'
[2] Injected with formalin.
[3] Figures in parentheses are ratios of organ to body weights.

The two tarsius (*Tarsius spectrum*) (table 5) are of especial interest since they belong to the most primitive type of primate and one which is rarely seen. (These figures on tarsius are included through the kindness of Drs. J. F. Fulton and H. D. Catchpole.) The female was known to be mature, the male probably very nearly so. They had been in the Laboratory for more than a year at the time of death. Both died as the result of filarial infestation.

b) Incomplete autopsies. In table 6 are included the brain and body weights of all monkeys of other species which had been examined at autopsy in this laboratory dur-

TABLE 4. BRAIN, ENDOCRINE AND ORGAN WEIGHTS OF THREE SPECIES OF MONKEY

Organ Weights

Species	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Right	Left				Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Mordax (Java)	3,400	7.40 (0.217)[3]	5.80 (0.17)[3]	14.55 (0.428)[3]	88.62 (2.60)[3]	5.59 (0.164)[3]	6.56 (0.193)[3]	6.67 (0.196)[3]
Rhesus hybrid	4,000	19.58 (0.489)	22.70 (0.567)	7.08 (0.177)	121.00 (3.02)	2.50 (0.0625)	11.03 (0.275)	12.09 (0.302)
Brown mangabey[1]	6,000	17.00 (0.283)	13.91 (0.231)	28.47 (0.474)	125.00 (2.083)	5.35 (0.0891)	9.92 (0.165)	10.42 (0.173)

Endocrine Weights

Species	Pituitary	Thyroid	Thymus	Pancreas	Adrenals		Ovaries		Testicles	
					Right	Left	Right	Left	Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Mordax (Java)	0.10 (0.00294)	0.55 (0.0161)	2.91 (0.0856)	4.59 (0.135)	0.34 (0.01)	0.47 (0.0138)			5.67 (0.166)	5.87 (0.172)
Rhesus hybrid[2]	0.08 (0.002)	0.58 (0.0145)	3.11 (0.0777)	5.07 (0.126)	0.54 (0.0135)	0.70 (0.0175)	0.15 (0.00375)	0.18 (0.0045)		
Brown mangabey[2]	0.28 (0.00466)	0.51 (0.0085)	6.95 (0.1158)	7.64 (0.127)	0.84 (0.014)	0.98 (0.0163)	0.40 (0.00666)	0.44 (0.00733)		

Brain Weights

Species	Sex	Maximum body wt.	Body wt., autopsy	Brain wt.	Brain wt. / Autopsy wt.	Died
		gm.	gm.	gm.		
Mordax[2]	M	3,400	3,400	92.00	2.70	Normal, killed
Brown mangabey[2]	F	6,100	6,000	88.85	1.48	Normal, killed

[1] *Cercocebus chrysogaster galeritus.*
[2] Mature.
[3] Figures in parentheses are ratios of organ to body weights.

ing the past 10 years together with the ratio of brain weight to body weight. Approximate age or maturity is given whenever possible. These are animals which did not have complete examination of all other organs, but whose brain and body weights were recorded either by the authors or by other observers in the Laboratory.

DISCUSSION

The significance of these data is discussed more fully in relation to the larger series of *Macaca mulatta*. There is little to be added here because the number of animals in each species is too small to be statistically significant. However, the same changes which occur due to sex, age or condition of health are indicated here also. Little can be learned from the brain weights beyond the actual figures except the consistent decreasing ratio between brain and body weights which appears in all species with growth.

The figures for the 17 sooty mangabeys show no sex or age differences because

TABLE 5. BRAIN, ENDOCRINE AND ORGAN WEIGHTS OF TWO TARSIUS SPECTRA

Organ Weights

	Body wt.	Lungs		Heart	Liver	Spleen	Kidneys	
		Right	Left				Right	Left
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Tarsius IV ♀[1]	168.0			0.635 (0.377)[2]	7.20 (4.28)[2]	0.031 (0.0184)[2]	0.72 (0.428)[2]	0.72 (0.428)[2]
Tarsius V ♂	112.0	0.92 (0.821)[2]	1.02 (0.91)[2]	1.20 (1.07)	7.10 (6.33)	0.128 (0.114)	0.683 (0.61)	

Endocrine Weights

	Pituitary	Thyroid	Thymus	Pancreas	Adrenals	
					Right	Left
		gm.	gm.	gm.	gm.	gm.
Tarsius IV ♀			0.168 (0.10)	0.56 (0.333)	0.15 (0.0892)	0.22 (0.13)
Tarsius V ♂		0.009 (0.00803)	0.05 (0.0446)	0.379 (0.338)	0.077 (0.0687)	0.076 (0.0678)

Brain Weights

	Maximum body wt.	Body wt. autopsy	Brain wt.	Brain wt. / Autopsy wt.	Died
	gm.	gm.	gm.		
Tarsius IV ♀	225.0	168.0	5.30	3.15	Parasitic worms, gastric hemorrhage
Tarsius V ♂	173.0	112.0	4.00	3.57	Parasitic worms

[1] The female was mature, the male nearly so.
[2] Figures in parentheses are ratios of organ weight to body weight.

TABLE 6. BRAIN AND BODY WEIGHTS OF 12 SPECIES OF NEW AND OLD WORLD MONKEYS

No. and sex	Species	Age	Body wt., maximum	Body wt., autopsy	Brain	Ratio Brain wt. / Body wt.
			gm.	gm.	gm.	
		Baboon				
43 ♀	*Papio hamadryas*	4 days	475	410	75	18.29
148 ♀		Mature	—	10,200	110	1.07
9 ♂		Mature	13,000	13,000	178	1.36
891 ♂	*Papio papio*	2 months	840	800	85	10.62
896 ♀		10 months	2,600	1,650	115	6.96
38 ♀			1,800	1,800	124	6.88
782 ♀		2 years	2,000	2,000	130	6.50
452 ♀			3,200	2,500	125	5.00
323 ♀			3,300	2,750	125	4.54
3 ♂			3,000	3,000	170	5.66
21 ♂			3,200	3,200	145	4.53
590 ♀			3,300	3,200	120	3.75
453 ♂			4,600	3,400	160	4.70

TABLE 6—*Continued*

No. and sex	Species	Age	Body wt., maximum	Body wt., autopsy	Brain	Ratio Brain wt. / Body wt.
			gm.	gm.	gm.	
			Baboon Continued			
170♀			3,500	3,450	120	3.47
448♀			3,500	3,500	135	3.85
450♂			4,200	3,800	130	3.42
454♀			4,500	3,800	148	5.28
8♂			4,200	4,120	163	3.95
P.4♀			4,600	4,200	110	2.62
187♀			4,500	4,500	140	3.11
449♀			4,800	4,800	130	2.70
170♂			5,600	4,800	142	2.95
167♂			4,900	4,900	160	3.26
14♀			5,450	5,450	170	3.11
188♂			5,650	5,600	170	3.03
32♀			7,800	6,000	140	2.33
654♀		Mature	6,000	6,000	135	2.25
1♀			7,800	6,050	138	2.28
4♀			6,800	6,800	138	2.03
4♂			7,050	6,970	163	2.33
370♀		Mature	11,800	7,300	210	2.87
529♀			8,040	8,000	194	2.43
15♂			8,150	8,150	194	2.39
653♂		Mature	8,400	8,400	160	1.90
12♂		Mature	8,850	8,500	152	1.78
842♀		Mature	12,100	8,600	237	2.75
11♀		Mature	—	9,500	187	1.96
24♀		Mature	12,450	9,700	142	1.46
109♀		Mature	11,600	10,800	130	1.20
27♂		Mature	10,800	10,800	180	1.66
09♀		Mature	—	13,700	145	1.05
31♀		Mature	14,380	14,380	205	1.42
30♀		Mature	16,650	14,500	180	1.24
2♂		Mature	17,600	17,600	160	0.91
25♂		Mature	21,800	21,800	193	0.89

Japanese Ape

No. and sex	Species	Age	Body wt.	Brain wt.	Brain wt. / Body wt.
			gm.	gm.	
255♀	*Macaca fuscata*	Mature	5,900	84	1.42

Erythrocebus

S.T.10	*Erythrocebus patas*		1,925	95	4.93
C.20♂			2,250	97	4.31
S.S.25			4,300	98	2.28
1246♀			4,600	100	2.17
S.S.22♂		Mature	4,900	118	2.40

Green Monkey

P.19♂	*Cercopithecus aethiops sabaeus*		2,300	60	2.61
3♂			2,600	65	2.50
756♀		Mature	3,000	55	1.83

TABLE 6—*Continued*

No. and sex	Species	Age	Body wt.	Brain wt.	Brain wt. / Body wt.
			gm.	gm.	
		Java Monkey			
♀	*Macaca irus*		950	38	4.00
G.M.10♀			1,300	45	3.46
N♀		Mature	1,400	50	3.57
V.3			1,850	65	3.51
P.T.7			2,075	55	2.65
D.4			2,285	55	2.40
C.C.12			2,700	37	1.37
F.4			4,500	70	1.55
371♂		Mature	4,700	70	1.49
		Mona Monkey			
S.T.2A	*Cercopithecus mona mona*		835	55	6.58
1196♂			1,400	55	3.93
		Spider Monkey			
6♀	*Ateles paniscus*		2,350	82	3.48
P7			3,750	92	2.45
5♂	*Ateles ater*		3,500	115	3.28
2♀		Mature	3,650	102	2.79
O3♂		Mature	3,900	93	2.38
T4♂		Mature	5,000	100	2.00
1♀	*Ateles grisescens*	Mature	4,160	80	1.92
P5♂	(red)		2,800	75	2.67
		Wooly Monkey			
2♂	*Lagothrix humboldtii*	Infant	1,600	82	5.12
1♂			3,800	75	1.97
5♂	*Lagothrix infumata*		3,800	95	2.50
4♀			4,000	95	2.37
		Cebus			
96♂			750	55	7.33
66♂	*Cebus fatuellus*		900	65	7.22
97♀			900	50	5.55
243			1,000	60	6.00
95♀			1,050	60	5.71
117			1,000	75	7.50
68♀		Mature	1,100	50	4.54
49♀		Mature	1,250	58	4.64
47♀		Mature	1,300	60	4.61
65♂		Mature	1,350	55	4.07
51♂		Mature	1,400	50	3.57
X		Mature	1,450	55	3.79
P8♀		Mature	1,600	65	4.06
69♀		Mature	1,650	65	3.94
P18♀		Mature	1,700	68	4.00
52♂		Mature	1,700	65	3.82
3♀	*Cebus azarae*	Mature	1,800	73	4.05
48♂		Mature	1,900	58	3.05
50♂		Mature	2,400	75	3.12

TABLE 6—*Continued*

No. and sex	Species	Age	Body wt.	Brain wt.	Brain wt. / Body wt.
			gm.	gm.	
		Potto			
2♀	*Perodicticus potto*	3 years	550	15	2.72
286♂		Mature	700	10	1.42
634♀		Mature	950	15	1.57
285♂		Mature	1,200	20	1.66
284♀			1,250	20	1.60
		Galago			
854♂	*Galago demidoffi*		95	4.8	5.05
447♀			155	3.7	2.38
		Marmosette			
5♂	*Aotus infulatus*		190	5.0	2.63
4♀	*Hapale humeralifer*	Mature	220	5.5	2.60
2	?		350	16.0	4.57
3♀	*Aotus infulatus*		510	14.5	2.84

they are nearly all immature animals. There is gradual increase in weight of all organs and endocrines with increase in body weight, except for the pituitary which changes little and the adrenals which increase relatively slightly.

Health, however, markedly affects the relationship of many organ weights to body weights. The 'normal' animals have much larger thymus glands than do those which showed disease or which had lost weight prior to autopsy, but the liver, and, to a less degree, the kidneys increase slightly under 'abnormal' conditions. Spleen and pancreas weights vary without relation to health, but are remarkably variable at any level of body weight. The left adrenal is constantly greater than the right. All these changes are found in *Macaca mulatta* and in anthropoids also.

SUMMARY

The brain, body, endocrine and organ weights are presented of a group of monkeys of various species which have been examined at autopsy in the Laboratory of Physiology of the Yale School of Medicine during the last 10 years. The data correlate with those compiled on a series of anthropoids and of *Macaca mulatta*. Full discussion of the findings are given in the paper on *Macaca mulatta*.

THE EFFECT OF THERMAL ENVIRONMENT ON THE MORPHOLOGY OF THE THYROID AND ADRENAL CORTICAL GLANDS IN THE ALBINO RAT[1]

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THAT neural mechanisms play the chief part in the control and integration of body temperature regulation seems firmly established (1–6). Recent developments, however, have indicated the existence of other accessory or subsidiary structures which play an important role in the evolution of a complete pattern of thermoregulation. Certain of the endocrines have been shown to be concerned with the maintenance of a constant body temperature, viz: the hypophysis (7, 8), the thyroid (9, 10), the adrenal medulla (11, 12, 13), and the adrenal cortex (12, 14–17). Endocrine participation in body temperature regulation is thus strongly indicated. In point of fact, the thyroid, by virtue of its control over the metabolic rate, and the adrenal gland (both constituent parts) have been linked together to constitute the most important non-neural accessory thermo-regulatory mechanism.

The effect of normal variations of external temperatures on thyroid morphology has been investigated in sheep and cats (18, 19, 20), in rats (21, 22), in teleosts (23) and in the sparrow (24). It has been found that there is a seasonal variation in thyroid activity as interpreted from morphological changes in the gland. These changes are stated to be due to seasonal variations in temperature.

The effects of experimentally altered environmental temperatures on the structure of the thyroid gland have been studied in rats and mice (25, 27–31), and in rabbits and guinea pigs (26). In general, it may be said that exposure to cold instigates increased activity on the part of the thyroid, which is indicated morphologically by heightened epithelium, resorption of intra-alveolar colloid and increased vascularity. Conversely, exposure to high temperatures inhibits thyroid activity, which is indicated, along with other changes, by a diminished epithelial cell height and intra-alveolar accumulation of colloid.

With regard to the adrenal cortex there is little evidence in the literature which illustrates a direct effect of season as such on the morphology of this portion of the gland. Zalesky (32), however, demonstrates that in the 13-lined ground squirrel adrenal cortical weight is a function of the sexual cycle, which in turn is dependent on the season of the year.

Variations in the amount of adrenal cortical lipoid with respect to age and sex have been indicated in mice (33), in guinea pigs (34) and in rabbits (35). Although references to the presence of cortical lipoid in the rat adrenal have been casually made, no attempt has apparently been undertaken to determine quantitatively the normal variation of this material and to correlate this figure with age, sex and season. A seasonal variation in this connection has already been indicated (36).

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The functional interpretation of morphological changes induced in the cortex as a result of experimentally altered thermal environments has been the subject of considerable controversy. This may be due partially to the different technical procedures employed by the various investigators. Cramer (12) indicates that high temperatures induce, among other changes, a more extensive deposition of cortical lipoid in the mouse adrenal. This has been confirmed in the rat (37, 17). Schmidt and Schmidt (31), on the other hand, have demonstrated that the lipoid-containing zone was narrowed under such conditions in the guinea pig. Flexner and Grollman (38) have shown that short exposures to heat caused an increase in osmic acid-reducing substances in all zones, while longer exposures resulted in complete loss of lipoid from all

TABLE 1. EFFECT OF THERMAL ENVIRONMENT ON THYROID AND ADRENAL INDICES

Group	No. animals	Temp. conditions (° F.) (10 days pre-autopsy)	Exper. procedure	Body temperature	Thyroid index, %		Adrenal index, %	
					Mean and σ_m	Range	Mean and σ_m	Range
Normal series								
N_1 (Jan.)	47	65° (constant)	—	Normal	50.6± 0.61	41.0–57.0	86.0± 1.1	71.0–100
N_2 (Mar.)	25 (90 days)	64° (rising)[1]	—	Normal	47.0± 0.42	43.6–52.0	93.0± 2.0	69.0–100
N_3 (May)	19	64° (rising)[2]	—	Normal	44.6± 1.1	38.2–55.0	98.6± 1.1	79.5–100
N_4 (June)	19 (90 days)	70° (falling)[3]	—	Normal	42.0± 1.1	34.0–50.0	87.0± 2.5	73.0–100
N_5 (Nov.)	24	65° (constant)	—	Normal	46.6± 0.59	42.0–53.0	81.0± 2.5	67.0–100
N_6 (Jan. '40)	5 (Rockland) 7 (Carworth)	65° (constant)	—	Normal	52.4± 0.78	49.0–58.0	63.6± 2.1	53.0–78.0
Exper. series								
Heat								
E_1	16	—	Artificial fever by high frequency 10 min./day for 10 days	102°F. each exposure	38.5± 0.95	31.0–44.0	100	—
E_2	10	—	Fever by oven at 107.6° 10 min./day for 10 days	102°F. each exposure	38.1± 0.46	34.0–44.0	90.1± 2.6	68.0–100
E_3	10	—	Prolonged oven fever at 91.4° for 10 days	103°F. continuously	42.3± 0.44	41.0–45.0	100	—
Cold								
E_4	10	—	Mild cold (53°) for 10 days	Normal	53.0± 0.66	51.2–57.0	69.8± 2.9	61.0– 72.0
E_5	15	—	Extreme cold (23.5°) for 2 days	Normal	56.1± 0.67	51.0–60.0	36.6± 3.9	10.0– 57.0
E_6	15	—	Extreme cold (32°) for 5 days	Normal	60.7± 0.98	56.0–66.0	67.7± 3.6	52.0–100
E_7	15	—	Extreme cold (32°) for 3 days; then 70° for 2 days	Normal	52.4± 0.70	49.0–58.0	63.5± 1.5	55.0– 75.0

[1] 36 to 49° av. outdoors.
[2] 52 to 61° av. outdoors.
[3] 75 to 68° av. outdoors.

portions of the cortex except the peripheral and inner fascicular zone. Exposures to mild cold for short periods, on the other hand, caused an increased reduction of osmic acid. This indicated the presence of more lipoid. With longer exposures to cold there was a marked loss of reducing substance.

Although the physiological aspects of temperature regulation through endocrine mediation have been under investigation for some time, there are still lacking detailed, correlated morphological data on the effect of thermal environment, including both normal seasonal as well as controlled experimental variations, upon these organs. With this in mind, the present series of experiments has been undertaken in order to determine the histological effects of different types of variation in the thermal environment on certain of the endocrines, specifically the thyroid and adrenal cortex; and to suggest the possible significance of these morphological changes in the maintenance of normal and experimental thermal economy of the warm blooded animal.

MATERIALS AND METHODS

The animals utilized (270) were albino rats, of both sexes and 60 days of age except where otherwise noted. The majority were from the same colony (Carworth

Farms) while a small number of experimental animals came from another source (Rockland Farms).

In all, 150 animals were used as a basic control series (*N*). These were divided into 6 groups, each of which was autopsied, after adequate acclimatization to laboratory conditions, at different seasons of the year. Laboratory temperatures were recorded by means of a recording thermometer.

One hundred twenty animals (series *E*) were divided into 7 groups, each of which was exposed to a specific alteration of environmental temperature (methods described in table 1). Rectal temperatures were obtained with a clinical thermometer. Both series had free access to food and water. The temperature data as well as conditions of exposure are indicated in table 1.

At the end of the test period, the animals were killed with ether, and the left adrenal was immediately removed and fixed in 10% formol; the right adrenal, gonad, thyroid, thymus and spleen were then removed and fixed in Susa (Heidenhain's modification). Particular care was exercised in handling the adrenals, to avoid bruising them with forceps; and in the case of the thyroids, the trachea, esophagus and surrounding structures at the level of the gland were dissected out and fixed as one piece so that the thyroid should not be damaged.

Formol-fixed adrenals were sectioned at 10μ in the longitudinal plane by means of the freezing microtome, and sections which passed approximately through the center of the gland were stained with Sudan III and counterstained with Harris' hematoxylin. Susa-fixed thyroids were prepared by routine paraffin methods, sectioned serially at 10μ and stained with triosin and Harris' hematoxylin.

To obviate subjective factors and to place the study on a more or less quantitative basis, weighed paper reconstructions were made. In order to determine the extent of the cortical lipoid, sections of the adrenal were projected onto paper, outlined, cut out and weighed by means of a chemical balance. The weight ratio $\frac{\text{lipoid-laden cortex}}{\text{whole cortex}} \times 100$ was obtained and an index for cortical lipoid, hereinafter referred to as the adrenal index, determined. Since the weights vary directly with the areas of the drawings of the figures, the value may also be considered as the area ratio. The adrenal index, however, does not take into consideration variation in concentration of lipoids. In sections of the adrenals of the normal rat, stained by standardized procedures, on the other hand, the differences in concentration of lipoids in the lipoid-laden portion of the cortex are relatively slight.

Similar procedures were followed in obtaining figures for the thyroid gland. Abnormal as well as peripheral alveoli were disregarded and 14 alveoli or more were outlined in each case. The weight ratio $\frac{\text{epithelial cell height}}{\text{whole alveolus}} \times 100$ thus obtained offers an index of epithelial cell height, which will subsequently be referred to as the thyroid index.

It was found by a careful survey of the data thus obtained that neither adrenal index nor thyroid index varies with sex. Hence, animals of both sexes could be included in the series without affecting the results.

The data concerning season and temperatures at the time of autopsy in the normal series and treatment in the experimental series are presented, together with average thyroid and adrenal indices in each group, in table 1.

GENERAL OBSERVATIONS

The animals of series *N* led an apparently normal existence during the laboratory tests. There were no untoward occurrences and all exhibited a normal weight gain. There was no marked variation in body temperature among the different groups.

In series *E*, heightened body temperatures, in the main, resulted in cyanosis of the extremities, respiratory distress, dehydration and disturbances in normal body weight

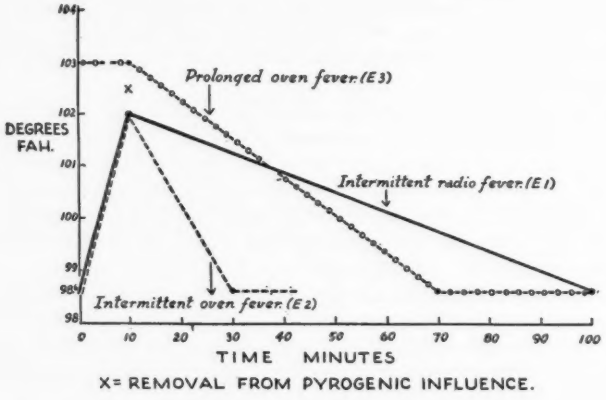

Fig. 1. Differential heating effects of various types of artificially induced fever. Intermittent radio fever produces the most persistent effect.

gain. These effects were most pronounced in *E*1 (intermittent radio fever) and least in *E*2 (intermittent oven fever). Some of the animals in *E*3 (prolonged oven fever) appeared lethargic and moribund at the end of the exposure period. Two of the animals died during the first night of exposure and the rest appeared to be in a state of flaccid shock. The rectal temperatures in *E*1 averaged 102.3°, in *E*2 102° and 102°F. in *E*3. These temperatures returned to normal 90 minutes after the end of treatment in *E*1, 20 minutes after in *E*2 and 60 minutes after in *E*3 (fig. 1). Upon autopsy, all of the heat-treated animals exhibited marked hyperemia and congestion of the viscera. This was most marked in the group subjected to radio fever.

Animals exposed to cold became active, the degree of activity depending on the temperature. Later they exhibited a tendency to huddle. The coats of animals exposed to extreme cold (*E*5) became shaggy and at autopsy the adrenals, which normally appear dark tan in color, were dull red and markedly enlarged. Some of the animals in this group, moreover, exhibited edematous and cyanotic posterior extremities. Two animals died during the first night of exposure when the temperature fell to 34°F. In *E*6 three animals died during the first night when the temperature fell to 18°F. Weight gain in this group was not normal. In *E*7 (cold to warm) similar conditions were noted but after a short stay in the laboratory (70°F.) edema and cyanosis disappeared. Two animals in this group died during the first night of exposure when the temperature fell to 22°F.

HISTOLOGICAL OBSERVATIONS

Thyroid

Normal series (*N*). Generally speaking, the picture presented by the thyroids of all the groups of animals in this series was approximately normal. Only under high

magnification could slight but significant differences be discerned. The epithelial cells in groups $N3$, $N4$ and $N5$ (summer) were of the cuboidal type, whereas in groups $N1$, $N2$ and $N6$ (winter) the cells were of the columnar variety. Thyroids of groups $N1$ and 6 exhibited the greatest number of colloid vacuoles, while in those of groups $N2$ and $N4$ colloid retraction was reduced to a minimum lending a plump, well-filled appearance to the alveoli. In only a few instances could desquamated epithelial cells

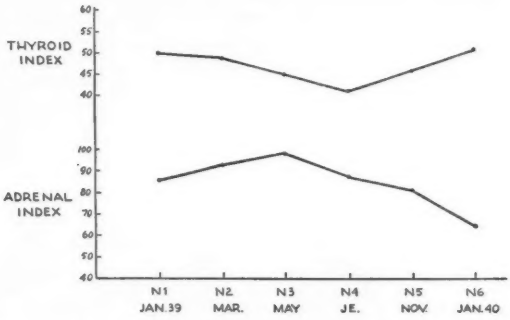

Fig. 2. Effect of season on thyroid and adrenal indices. Note that while adrenal index varies with normal rises in temperature ($N2$ and $N3$) and with normal falls ($N4$), thyroid index does not vary correspondingly. In the winter months thyroid index is high while adrenal index is low, whereas in the summer months this situation is reversed. Note also that ordinate units are not comparable in the two instances. Thyroid index is plotted over wider intervals than adrenal index because of the relatively slight variations in the former.

be discerned in the alveoli ($N2$). There was no marked variation in vascularity al though there was an indication of a slight increase in groups $N1$ and $N6$. Similarly, there was no marked variation in distribution of interfollicular cells in any of the groups.

Experimental series (E), *heat treated animals.* Although there were minor differences, in the main, the histological picture was essentially the same in the various heat treated groups ($E1$, $E2$ and $E3$). The nuclei of the very low cuboidal lining epithelial cells were flattened and the cell boundaries were more or less indistinct (fig. 3). This, however, was not a universal condition. Interfollicular capillaries were markedly congested with erythrocytes. The most marked variations were noted in sections of group $E3$ (prolonged oven fever). Here the lining cells were markedly flattened and the alveoli greatly distended with deeply eosinophilic colloid. Peripheral colloid vacuoles were not apparent in some of the alveoli, but in others of this group vacuolization was very pronounced.

Cold treated animals. The thyroids of animals exposed to cold revealed more or less the same characteristics in all groups: low columnar lining epithelium, distinct cell boundaries, large and hyperchromatic nuclei and irregularly shaped and smaller alveoli. In group $E4$ marked peripheral colloid vacuolization, but no retraction, was discernible. In all of the animals, the affinity of the colloid for eosin was noticeably diminished. Interfollicular vascularity was increased. The glands of group $E7$ (cold to warm) however, appeared normal.

In both heat and cold treated animals, interfollicular cells were present, and no striking difference in distribution between the various groups could be noted. Only occasionally could desquamated cells and leucocytes be discerned in the alveolar colloid, and then only in some of the alveoli.

Adrenal cortex

The normal adrenal cortical section stained with Sudan III and Harris' hematoxylin is best studied under the 16 mm. objective. High power work is difficult owing to the refractive nature of the sudanophilic material in the cortical cells. A detailed description of the normal rat adrenal stained in this manner has previously been reported (17, 37).

Normal series (*N*). In the main, adrenals of all groups in this series presented a

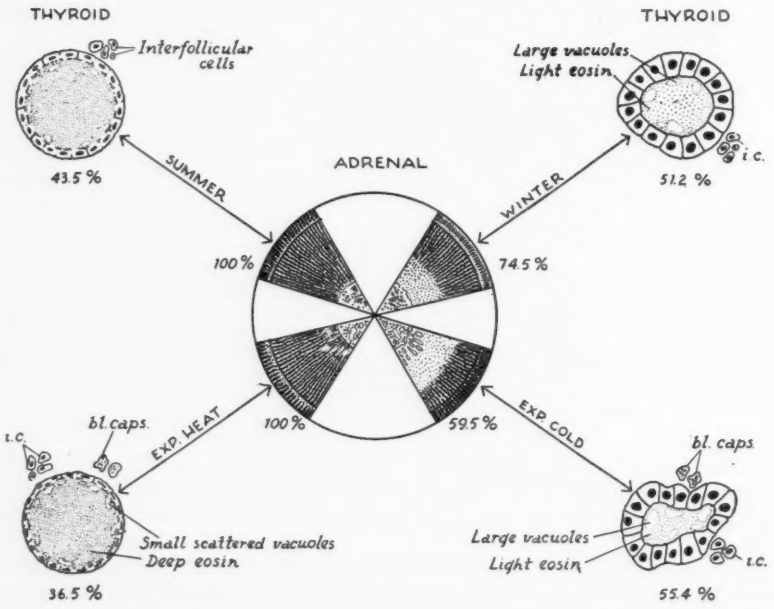

Fig. 3. Effect of 1) season and 2) experimentally altered environmental temperatures on the morphology of the thyroid and adrenal glands. The figures indicate average indices in each instance.

Note the flattened epithelium, light colloid and indistinct cell boundaries in the summer thyroid, as well as the extensive distribution of adrenal cortical sudanophilic substance. Winter thyroids, on the other hand, show heightened epithelium, large hyperchromatic nuclei, distinct cell boundaries and large peripheral colloid vacuoles; adrenals here exhibit a distinct lipoid-free zone in the reticularis.

In experimentally heated groups the epithelium of the thyroid is greatly flattened and colloid is deeply eosinophilic; the adrenal cortex exhibits an extensive deposition of sudanophilic substance and markedly dilated cortical and medullary sinusoids. The thyroids of cold treated groups show heightened epithelium, faintly eosinophilic colloid with large peripheral vacuoles and smaller and more irregular alveoli. Blood capillaries here, as well as in the experimentally heated groups, are engorged with blood cells. Adrenals of cold treated groups show a marked diminution of cortical sudanophilic substance and increased vascularity.

more or less normal picture. There was, however, a marked variation in the extent of distribution of the cortical lipoid among the various seasonal groups, and, moreover, between the individuals within the several groups. This variation is shown in table 1 and represented in figure 2. No significant variation in cortical vascularity could be detected in any of the groups in the *N* series.

Experimental series (*E*), *heat treated animals.* There were slight histological differences among adrenals in groups E_1, E_2 and E_3 but all exhibited a markedly increased extension of the cortical sudanophilic substance toward the medulla in such a manner

as apparently to mask the normally lipoid-free zona reticularis. This situation applies only if the temperature is relatively high, and most important, maintained over a long period of time, since it has been shown (17, 37) that single lethal exposures to heat have little or no effect on the distribution of cortical lipoid. The primary effect here is a marked dilatation of the cortical and medullary blood vessels.

Cold treated animals. All cold treated animals revealed increased vascularity in adrenal sections. The most marked effects here, as in the heat series, were variations in distribution of cortical lipoid. Sections of the adrenals of these animals, especially those of groups *E5* and *E6*, were noticeably larger in size than normally. This enlargement, however, had no effect on adrenal index, since measurement of this factor was on a relative basis. Group *E4* revealed the greatest variation in distribution of cortical lipoid among individual animals. Furthermore, two animals in this group exhibited an apparently reversed lipoid distribution, i.e. there was no sudanophilic substance in the outer cortex but instead the lipoid had assumed the position of a narrow band located in the inner reticular zone (which is normally free from lipoid) encircling the medulla.

The morphological variations (normal and experimental) in both the thyroid and the adrenal are illustrated semi-diagrammatically in figure 3.

DISCUSSION

From the evidence presented in these experiments, it is apparent that environmental temperatures exert an influence upon the morphology of both the thyroid and adrenal cortex, and further, that these constituents of the endocrine system may, in some manner, be concerned with the process of bodily thermoregulation, since exposures to environments which tend to alter body temperature induce morphological variations in these organs.

In seeking an etiological factor for the observed seasonal variation in the thyroid, it may seem, at first glance, that variation in temperature as such is the causative agent involved. That such a view, however, cannot be maintained, is evident upon careful examination of the average laboratory temperatures to which the normal (*N*) series of animals had been exposed. Reference to table 1 will show that these temperatures during the various months were approximately the same in all instances. For example, during the winter months the laboratory temperature averaged 65°, but this was not much lower than the recorded summer temperature, which averaged 70°. The thyroid index, however, averaged 42% for the June group and 51.5% for the winter groups. Statistical treatment of the figures shows this difference to be mathematically significant.

It appears, therefore, that normal seasonal temperatures play no significant role in altering thyroid morphology. This differs from the view expressed by Miller, Kenyon and others, who believed that seasonal morphological variations were directly related to differences in environmental temperature. The results of the present investigations indicate that under normal temperature conditions there is a seasonal variation in thyroid morphology which apparently bears no direct relationship to normal laboratory temperatures. However, abnormal temperature variations which tend to alter body temperature, as those used in the experimental (*E*) series, produce distinct effects on thyroid morphology. These are essentially in accord with previously recorded observations.

A possible cause of the observed seasonal variations in thyroid as well as adrenal index may be based on the amount of physical activity of the animals, which apparently varies considerably during different times of the year. The rat is more or less a nocturnal animal and with the shortened daylight hours of winter is apt to be more

active during this time. Unfortunately, however, no quantitative indication of activity during the summer and winter months was obtained and the cause of the seasonal variation is thus still obscure.

To test the role of extremes in thermal environment in bringing about alterations in glandular morphology the *E* series of experiments was planned and carried out. Reference to figure 4 will indicate that an inverse relation exists between extremes in temperature and thyroid index, and that the two factors are related in a somewhat quantitative fashion. Increasing intensity and duration of exposures to abnormal cold result in an almost step-like increase in thyroid index. Conversely, increased heating causes a decrease in the index. Intermittent doses of high frequency fever (*E*1) induced the relatively low index of 38.5%, which was considerably lower than the lowest seasonal index (42% in 4). The intensity of the treatment in this particular experiment may be noted by reference to figure 1 which indicates that the artificial fever

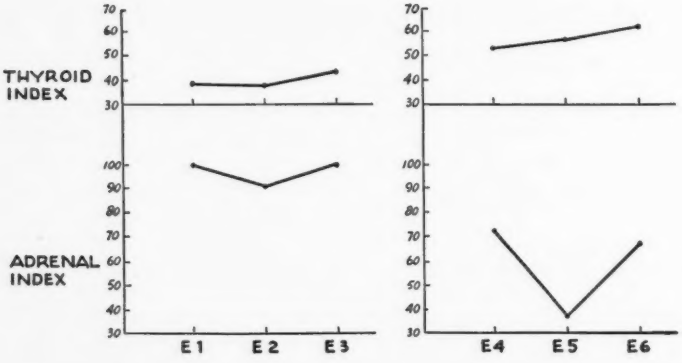

Fig. 4. Inverse relation between thyroid and adrenal indices upon subjection of animals to experimental variations in thermal environment. Increasing intensity and duration of cold results in a cumulative effect on thyroid index, but adrenal index as a result of prolonged exposure to cold, tends to revert to normal (*E*6). (*E*1, intermittent high frequency fever; *E*2, intermittent oven fever; *E*3, prolonged oven fever; *E*4 prolonged mild cold; *E*5, extreme cold; *E*6, prolonged extreme cold.)

induced by this means persisted for a longer period of time than that of the oven induced type.

It appears then, that changes in environmental temperatures which tend to alter body temperature play an important part in varying thyroid morphology. The effects of abrupt changes in temperature and of the duration of exposures are illustrated by groups *E*5, *E*6, *E*7 and their control, *N*6. All of these animals were from the same colony and were autopsied in the same month (January '40). Group *E*6 was exposed to a constant freezing temperature (32°) for 5 days, *E*5 was placed outdoors for two days (23.5°), and the animals in *E*7 were kept outdoors for 3 days (32°), and not autopsied until they had been in a warm room (70°) for 2 days after the exposure. When the variations in temperature and thyroid index were plotted (fig. 5) a clear-cut result was obtained which may be accepted as evidence of the reversibility of the phenomenon of morphological variation. This is true, however, only if no damage has been inflicted upon the gland. More significant, however, is the fact that prolonged extremes of temperature in either direction cause a progressive, constant effect on the thyroid index which ap-

pears additive in nature. Short exposures cause similar variations in a given direction, but not to such a marked degree. It seems, then, that morphological thyroid variations are inversely related to observed experimental temperatures, and that these variations are in direct relation to the intensity and duration of the thermal stimulus.

It is possible that abnormal situations which tend to lower body temperature cause a compensatory hyperactivity as indicated by the morphological picture. Exposure to experimental heat, a procedure which tends to raise the body temperature, on the other hand, induces the opposite morphological picture. Flattened epithelium (low thyroid index) and concomitant accumulation of intra-alveolar colloid may indicate a state of relative inactivity of the gland. The relation of morphology to activity becomes more difficult to interpret in the latter instance, since expression of

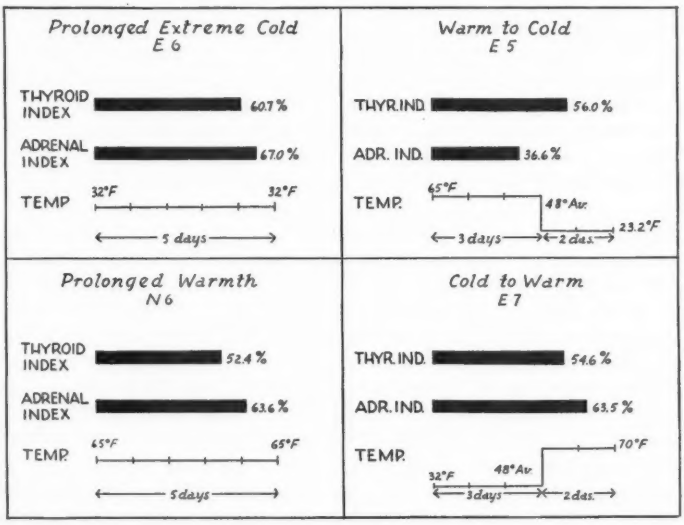

Fig. 5. Effects of changes in thermal environment on the thyroid-adrenal cortical apparatus. The figures for group E_7 demonstrate the reversibility of the thermal effect on the two endocrines. N_6 is the control group for this series.

glandular activity here may be only relative. It is entirely possible that the epithelial cells are actively secreting into the alveolar lumen while none of the active principle is leaving the gland (i.e, a storage phase). On the other hand, increased body temperatures may decrease the need for thyroid principle by the tissues, and, as a result the gland stores its secretion in the form of colloid. Continued accumulation of this substance would cause increased pressure upon the lining cells, which, as a result, would assume a slightly flattened configuration. Thus, flattened epithelium and colloid distension may still represent a very "active" gland with respect to the character of the epithelial cell. The general consensus of opinion, however, seems to be that heightened epithelium indicates hyperactivity while a flattened epithelium denotes a hypoactive state (12, 22, 28).

Epithelial cell height, however, in heat treated animals may still be safely utilized as a criterion of thyroid function, albeit an indirect one, since in the last analysis, the quantity of active principle leaving the gland is the determining factor with respect

to glandular activity. Whatever the situation may be, it can be safely assumed that the thyroid index follows experimentally altered temperatures in an inverse direction and that this may reflect an attempt on the part of the normally thermoregulating organism to maintain thermal equilibrium.

It is to be understood, moreover, that no claim is being made for a direct and isolated action in this respect. The other heat regulating mechanisms, especially the hypothalamico-hypophyseal system, may also be involved. It is entirely possible that the thermal effect is an indirect one acting primarily through the heat regulating centers of the hypothalamus or the anterior hypophysis, and that the morphological variations observed may be due to an indirect effect on the thyroid through the thyrotropic principle of the latter. The changes in the adrenal cortex, similarly, may be affected by corticotropic influence of the anterior hypophysis. At any rate, no matter what the exact mechanism, direct or indirect, extremes in thermal environment exert an effect on glandular morphology.

From the observations made here, no definite significance could be assigned to the interfollicular cells. It has been claimed (30) that these elements greatly increase in number and give rise to new follicles if the need for thyroid principle is increased, i.e., upon exposure to cold. Although detailed cell counts were not attempted in the present study, no marked variation in the number and distribution of these cell groups could be discerned among the various groups (both normal and experimental) of animals.

The results of this study also indicate that the effect of generalized fever induced by ultra-high frequency radio emanations is apparently no different from that of fever caused by externally applied heat, with regard to thyroid and adrenal cortical morphology. In the thyroid, flattened epithelium, colloid distension and hyperemia are brought about by application of the two totally different types of thermal stimuli. In the case of radio-induced fever, however, the effect is apparently more intense and persists over a longer period of time (fig. 1). This is in accord with previously reported observations and it would appear that ultra-high frequency radio emanations cause no specific effect upon the tissues other than those brought about by the induced rise in body temperature.

The relatively increased vascularity in animals exposed to radio emanations as compared to externally applied heat may be caused by the difference in rate and intensity of heating. This is in accord with the beliefs of Kahler et al. (39) and Bevelander and Bernstein (40).

The adrenal cortex, in contradistinction to the thyroid, is an extremely sensitive organ, whose morphology may be altered considerably by a wide variety of stimuli. Its most striking feature is the marked variation, under both normal and experimental conditions, of the distribution of the sudanophilic substance. In fact, the situation is such that it is difficult to cite a 'normal' adrenal index. The figures, as can be noted from table 1, indicate an extremely wide divergence between, as well as within the various groups of animals.

That the cortical index may vary with the season is readily confirmed here. The obvious fact to be noted from the present investigation, however, is that, unlike the thyroid index, the cortical lipoid varies with changes in temperature which may be considered normal. This tends to skew the seasonal curve (fig. 2) of the cortical index out of line. For example, the outdoor temperature of the 10-day preautopsy period, in the March group (N_2), was a rising one: from 36° to 49°F.; the adrenal index here was 93%. In the May group (N_3) the temperature was likewise a rising one, but on a higher absolute level (52° to 61°F.); the adrenal index here apparently followed the increasing temperature since the figure was 98.6%. On the other hand, in the June

group ($N4$) although the average temperature was on a still higher absolute level (70°F.), there was a progressive fall in outdoor temperature which was reflected in the laboratory temperature (75° to 68°F.) during the pre-autopsy period; the adrenal index here dropped to 87%. Significantly enough, the thyroid index exhibited no such variation. This would tend to enhance the view that the adrenal cortex is more readily affected by normal variations in environmental temperatures. The apparent discrepancy for the divergent adrenal values in the two January groups ($N1$, 86% and $N6$, 63.6%) may be partly due to the fact that these groups were unavoidably obtained from different colonies.

Reference to figure 4 will indicate that application of any form of thermal stimulation which tends to raise body temperature (groups $E1$, $E2$ and $E3$) causes an increased deposition of sudanophil substance (increased adrenal index) to 100% in almost all cases, whereas exposures to extreme conditions which tend to lower the body temperature, cause a marked decrease in the cortical distribution of this substance (lowered adrenal index).

Exposure to cold results in an apparently more complicated set of conditions than exposure to heat. It will be noted (fig. 4) that prolonged mild cold (10 days at 53°F.) induced an adrenal index of 69.8% in group $E4$. Exposure to severe cold (23.5°F.) for a short period (2 days) caused a precipitous drop to 36.6% in group $E5$, while prolonged exposure to extreme cold (32°F. for 5 days) brought the index back up again to 67.7% in group $E6$. It is interesting to note that the last-mentioned treatment actually caused the index to exceed the figure for the controls for this group ($N6$) which was 63.6%. This apparent inconsistency may very well be explained on a basis of compensatory action on the part of the adrenal cortex. The lengthened period of exposure in this instance may afford time for the cortex to become accustomed to the secretory strain and would allow for a renewal of the store of sudanophilic substance. This may also be indicative of the greater variability in cortical response to thermal environment.

Still further indication of the cortex in this regard is illustrated by the fact that the index (63.5%) in group $E7$ (3 days of cold followed by 2 days of warmth) reverted to a value almost identical with the figure for the controls of this group (63.6%). It may be noted (fig. 5) that thyroid index in $E7$ has not, in contradistinction to the cortex, returned to the normal figure. This may also be accepted as evidence of the reversibility of the phenomenon. It becomes evident, therefore, that relatively short exposures of extremes in environmental temperatures may profoundly affect the distribution of cortical sudanophilic substance. The rapid response of the cortex in this regard, it would appear, confirms Cramer's conception of the 'explosive' nature of cortical response to abnormal temperature variations. The apparent inconsistency in group $E6$ has already been explained on the basis of compensation dependent on a time factor.

These experiments, then, amply support the premise that the lipoid of the adrenal cortex is readily affected by changes in absolute environmental temperatures as well as by rates of change. In this respect the cortex is more sensitive than the thyroid, since under equivalent temperature conditions, greater variation is apparent in adrenal than in thyroid indices.

The functional significance of the morphological appearance of the adrenal cortex with regard to distribution of sudanophilic substance at any particular time is subject to various interpretations. This is a situation commonly encountered in morphological endocrine studies generally, particularly in the case of the adrenal cortex. Whether increased deposition of sudanophil substance, observed after exposure of animals to conditions which tended to raise body temperature, indicates an increased secretion phase or merely an increased storage phase (inactive) analogous to that found in the

thyroid, cannot safely be stated. Although Cramer's contention that spread of cortical lipoid indicates hyperactivity seems quite probable, the possibility that the condition indicates a storage phase (41) should not be entirely ignored.

At any rate, it is demonstrated that exposures to continued mild elevations of body temperature cause a pronounced deposition of sudanophilic substance in regions which normally contain little, if any, of this material. The presence of considerable amounts of lipoid in the zona reticularis of a small number of normal winter animals is, in all probability, a reflection of the normal variation, which, as has already been indicated, may be considerable. Only a relatively small number of winter adrenals exhibited sudanophilic substance in this zone, whereas the incidence of the condition in the summer and experimentally heated groups was much higher.

The appearance of sudanophilic material in the reversed position in animals exposed to short intervals of extreme cold (E_5) is, however, of quite a different nature. The severity of this treatment is indicated by the fact that 2 animals succumbed to its effects. Here, the only site of sudanophilic deposition is a thin band in the juxtamedullary reticularis. This may be indicative of a cortex on the way to exhaustion. In no instance, however, was any gland completely devoid of this substance. Before such a condition is reached, the cortex in a normally heat regulating animal may enter upon a compensatory phase and the adrenal index starts on its way back to the normal condition.

Whatever the situation concerning the physiological aspects of cortical activity may be, it appears clear that heightened environmental temperatures affect adrenal index in one direction, while lowered temperatures produce an opposite effect. It is interesting to note, furthermore, that the variations in cortical lipoid in a given direction occur no matter what the heat inducing agent may be. Rises in body temperature to a comparable level, i.e., those induced by intermittent doses of radio fever (E_1), intermittent doses of oven induced fever (E_2) and prolonged oven induced fever (E_2), all bring about a similar condition in the distribution of cortical lipoid. This is evidence that the adrenal cortex (like the thyroid) is affected by a generalized temperature effect and not through a specific action by any of these thermogenic agents.

Objection may be raised, in view of Selye's findings (42) in connection with the role of the adrenal cortex in the so-called 'alarm reaction', to the results obtained in the experimentally heated animals. It is the contention of this investigator that, among other changes, the adrenal cortex responds by hypertrophy to non-specific alarming stimuli. No claim is made in the present investigation that extremes in temperature, which must of necessity fall into the category of alarming stimuli, specifically affect the cortex. In point of fact, the generalized effect of radio induced artificial fever has already been emphasized. Moreover, if extension of cortical lipoid is due to an 'alarm reaction,' on what grounds can the adrenal index figures revealed by the normal summer adrenals (almost 100% in all groups) be explained? Here, normal summer temperatures, albeit relatively high, can hardly be construed as constituting an 'alarming' stimulus. It would seem rather, that the state of the cortex here represents a normal adaptation to a sub-critical variation of environmental temperature.

As to the underlying physiological mechanism of the experimental variations in the adrenal as well as the thyroid index, relatively little can be said with safety. One possibility is altered tissue metabolism brought about by the thermal variations. These metabolic changes may then make their effects manifest upon the morphology of the two organs. Another possibility is a disturbance in salt and water metabolism as a result of extremes in temperature. It is well known that the adrenal cortex is vitally concerned with these two factors (43). It is also possible that the thyroid and the adrenal cortex are concerned in the thermoregulatory process of the warm-blooded

organism, and that the morphological changes observed are actually responses to conditions which tend to alter normal bodily thermal equilibrium. The exact physiological mechanism for such action, however, is still obscure.

SUMMARY

Evidence is presented to indicate a normal seasonal variation in epithelial cell height in the thyroid (high in winter and low in summer) and in the distribution of the adrenal cortical sudanophil substance (low in winter and high in summer) in the 60- and 90-day-old albino rat. These seasonal variations apparently cannot be correlated with environmental temperature.

While the thyroid index more or less rigidly follows a seasonal curve, the adrenal index is more likely to vary with changes in the thermal environment which may be considered normal.

Extremes in environmental temperature which tend to alter body temperature are causative factors in producing morphological variations in these two organs. Epithelial cell height is inversely related to experimentally altered environmental temperatures, whereas distribution of cortical sudanophilic material is directly related.

Evidence is adduced to illustrate that as far as the thyroid and the adrenal cortex are concerned, the morphological effect of artificial fever induced by ultra-high frequency emanations differs in no great respect from that induced by externally applied oven heat.

Morphological variations in both the thyroid and the adrenal cortex induced by experimentally altered thermal environments are reversible provided no tissue injury has been sustained.

The thyroid and the adrenal cortical glands are, in some manner, concerned with the maintenance of a constant body temperature and may operate in conjunction with the neural thermoregulatory mechanisms.

The author wishes to acknowledge the gracious assistance of Professor M. M. Hoskins, under whose supervision this study was undertaken. Further thanks are due the Research Laboratories of the General Electric Company for the generous provision of certain of the technical equipment utilized in the investigation.

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NOTES AND COMMENTS

EFFECT OF TESTOSTERONE PROPIONATE AND ESTRADIOL DIPROPIONATE ON THE CHOLESTEROL CONTENT OF THE BLOOD AND AORTA OF RABBITS

THE EFFECT of sex hormones on cholesterol metabolism has been the subject of several recent investigations. Entenman, Lorenz and Chaikoff[1] noted increases in the blood lipids of male and female birds following the administration of estrin; these observations have been amply verified by other workers using various estrogenic substances.[2,3] While the present studies were in progress, Entenman, Lorenz and Chaikoff[4] reported that the blood lipids of birds were not affected by the administration of large doses of testosterone, progesterone and desoxycorticosterone acetate. Although Blinoff[5] found no changes in the cholesterol content of the blood of dogs before and after castration, Kochakian et al.[6] observed high plasma lipid values in fat castrates and normal values in thin castrates (dogs). Furthermore, Kochakian and his coworkers noted no change in the plasma lipids of normal or castrated dogs after injections of androstenedione for short periods of time.

In view of these findings and because of the chemical similarity of cholesterol to the steroid hormones, it appeared desirable to determine the effects of the prolonged administration of some of the commonly employed steroid sex hormones on the cholesterol metabolism of rabbits. This animal was chosen for this study since the rabbit is known to be very susceptible to factors influencing lipid metabolism.

Materials and methods. Twenty-four young male and female rabbits, 3 to 5 months old and weighing from 1.6 to 2.5 kg., were used. The animals were kept in individual cages and were fed measured amounts of Ralston-Purina Rabbit Chow daily. Testosterone propionate and estradiol dipropionate* were administered intramuscularly in dilutions so adjusted that each dose was dissolved in 1.0 cc. of sesame oil. Four male and three female rabbits received 10 mg. of testosterone propionate and 5 males and 4 females received 0.2 mg. estradiol dipropionate daily during the first week and 3 times weekly during the subsequent 13 weeks of the experiment. Of the 8 control rabbits, 2 males and 2 females were given 1.0 cc. of sesame oil in the same sequence and 3 males and one female received no injections.

The whole blood cholesterol was determined every 8 to 16 days by a modified Bloor method.[7,8] The procedure used for killing the rabbits, the technic for the removal of the aorta and the determination of its cholesterol content have been described elsewhere.[9]

Results. The results are given in table 1. In general, it will be observed that female rabbits, both control and experimental, have higher concentrations of cholesterol in blood

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* The testosterone propionate (Perandren) and estradiol dipropionate (Di-Ovocylin) were supplied by the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

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TABLE 1. THE CHOLESTEROL CONTENT OF THE BLOOD AND AORTAS OF MALE AND FEMALE RABBITS RECEIVING SESAME OIL (*Group A*), TESTOSTERONE PROPIONATE (*Group B*) AND ESTRADIOL DIPROPIONATE (*Group C*)

	Rabbit No.	Sex	Blood cholesterol (mg. %)										Aorta cholesterol
			Days										
			0	8	21	35	49	65	76	90	100	Mean	
													mg./ 100 gm.
	70	M	84	84	101	103	77	78	122	93	89	92.3	495.0
	48	M	70	70	88		76	103	142	145	82	97.0	534.0
	15*	M		113	92		85	112			89	98.2	750.0
Group A	16*	M		76	82		83	84			111	87.2	360.0
	17*	M		87	86		83	93			105	90.8	360.0
Control	Average		77	86	90		81	94	132	119	95	93.1	499.8
	26	F	65	68	60	67	80	90	99	105	92	80.7	520.7
	80	F	126	134	128	172	213	173	244	264	315	196.5	567.2
	21*	F		126	65		114	146				112.8	460.0
	Average		95	109	84	120	136	136	172	184	204	130.0	516.0
	65	M	81	74	78	85	76	76	92	79	59	77.7	384.0
	66	M	98	80	62	85	84	80	92	105	52	82.0	430.6
Group B	67	M	95	83	69	77	113	108	95	91	60	87.9	479.6
	81	M	44	96	100	77	89	86	80	89	82	82.5	411.4
Testosterone propionate	Average		80	83	77	81	91	88	90	91	63	82.5	426.4
	38	F	106	105	104	221	107	121	109	96	74	115.9	513.2
	73	F	110	105	101	78	109	108	112	78	62	95.9	472.7
	75	F	75	107	119	95	97	88	113	79	73	94.0	508.7
	Average		97	106	108	131	104	106	111	81	70	101.9	498.2
	51	M	76	75	81	86	100	76	78	118	121	90.1	401.1
	68	M	77	89	92	90	93	108	107	110	103	96.5	442.8
Group C	69	M	83	87	89	82	76	94	173	129	91	100.4	533.7
	96	M	74	90	99	110	126	129	172	157	159	124.0	443.1
Estradiol dipropionate	71	M	69	90	99	109	85	92	97	92	98	92.3	584.7
	Average		76	86	92	95	96	100	125	121	114	100.7	481.1
	37	F	74	65	112	163	140	143	155	180	193	136.1	591.6
	76	F	77	89	114	126	168	162	177	156	162	136.8	569.7
	77	F	104	94	102	128	120	99	108	107	128	110.0	431.9
	78	F	86	94	116	120	128	120	136	110	127	115.2	521.6
	Average		85	85	111	134	138	134	144	138	148	124.5	528.7

* Received no injections.

and aorta than do male rabbits. However, this protocol reveals that testosterone propionate and estradiol dipropionate are relatively inert as regards their ability to alter the cholesterol content of the blood and aorta of rabbits. Although the average blood and aorta cholesterol of both the male and female animals receiving testosterone propionate are somewhat lower than those observed in the control rabbits, the difference is not statistically significant.

Discussion. Although various estrogenic substances have been shown to elevate the blood lipid value of birds,[1,2,3,4] the present studies fail to reveal any appreciable changes in the cholesterol content of the blood or aorta of rabbits after the administration of a total of 9 mg. of estradiol dipropionate given in divided doses over a period of 100 days. The amount used was smaller per unit body weight than those employed by some of the other investigators, although it must be conceded that this dose is large for a rabbit and represents an amount

many times greater than that generally employed for human subjects. Profound secondary sexual changes were noted as evidence of the effectiveness of the dosage used.

Although this study reveals no significant changes in the cholesterol content of the blood or aorta of male and female rabbits after the administration of testosterone propionate it is of interest that McCullagh, McCullagh and Hicken[10] reported a tendency for the blood cholesterol to decrease during the injection of androgenic urinary extracts in hypogonadal and eunuchoid patients. This trend was especially marked in fat eunuchs with high blood cholesterol values. Teilum[11,12] reported a gradual increase in blood cholesterol after castration although Kearns[13] observed a decrease in the blood cholesterol of castrated men. Randall[14] has recently reported a slight increase in the blood lipids during a 3-week course of injections of testosterone propionate in nine schizophrenic subjects, although he states[15] that in 4 normal subjects no appreciable variations in blood lipid values were noted.

In work being carried out at present in this laboratory, testosterone and estradiol appear to inhibit hypercholesterolemia and atherosclerosis of the aorta in female rabbits but not in male rabbits fed cholesterol. This will be made the subject of a later report.

Summary. The administration of 450 mg. of testosterone propionate or 9 mg. of estradiol dipropionate in divided doses over a period of 100 days failed to produce any noteworthy changes in the cholesterol content of the blood or aorta of young male and female rabbits.

Grateful recognition is given to Julius Wiland, B. S. for valuable technical assistance.

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HISTOLOGICAL EFFECTS OF INJECTING RESIDUAL OVARIAN EXTRACTS*

THE PREPARATION and some physiological effects of injecting residual ovarian extracts are described in previous communications.[1-4] In one of these[1] it was mentioned that a histological examination showed more embedded ova in the experimental animals than in the controls. It is the purpose of this paper to analyze more in detail the histological differences and to show that these differences may be due to the reciprocal action of the ovarian extract upon the pituitary gland.

Young immature (21–23 days old) rats, were injected with residual ovarian extract for 10 to 20 days. The animals received in general ¼ cc. of the extract equivalent to 3.6 gm. of fresh ovary daily, which contain estrogenic substances equivalent to 1 R.U. per day. It took 5 R.U. per day of estrone[1] to open the vagina in the same time. All animals were killed by

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ether, the body cavity opened immediately and the ovaries removed, fixed, sectioned and stained as soon as possible.

The histological effects of the experiment were recorded by one of us (F. G.) and then verified by Dr. George Johnson. These results are summarized in table one. Atresia was prevalent throughout the entire group of animals. There appeared to be more atresia in the larger follicles of the experimental ovaries than in the control animals. The one constant difference was found to be in size of the follicles open or partially open (table 1). The open

TABLE 1. SUMMARY OF HISTOLOGICAL RESULTS[1]

	Controls	Experimentals
Atretic follicles		
Small to medium	Many	Many
Medium to large	Several	Several to many
Open follicles		
Small	26.4μ	25.7μ
Large	41.3μ	41.6μ
Partially open follicles		
Large	27.9μ	38.1μ
Closed follicles		
Small	23μ	
Large		32.6μ
Interstitial tissue		
	Medium	Abundant

[1] From an unpublished M.S. thesis by one of us (F. G.) and used by the permission of the Department of Zoology of Kansas State College.

follicles of the experimental ovary were further characterized by having a thick granulosa. Animal A 161 had the largest follicle, 80 mμ. in diameter, and the wall showed a strong tendency to luteinize. There was more interstitial tissue to be observed in the experimental animals.

The ovary of the normal 21-day-old rat is almost entirely composed of small follicles. Little difference can be noted in the 21 and the 36-day-old rat which was the age at which most of the animals were killed. The principal difference being that there were more follicles in the older ovary. However, in the experimentals the follicles were consistently larger and more open or partially open. The walls of these follicles were thicker than the ones from the control ovaries.

The histological studies of the sectioned uteri confirm an earlier report of more thickened walls of the experimentals. The epithelial tissue was highly columnar in the experimentals.

The histological observations made on animals which were in almost complete estrus but still well within normal variation as reported by Long and Evans[5] tend to discount the idea that estrone and estriol are the active substances. They do not cause the increase of uterus size comparable to estradiol.[6] Since all the experimentals have larger follicular growth it seems that the anterior pituitary was stimulated by the extract. Since atresia is about the same in both groups of animals and is a natural characteristic of young growing ovaries, we have concluded that evidence points to another active substance[6] causing these histological changes reported since estrone inhibits the ovary. As cited in the paper[1] above, mathematically at least, there could not be enough estrogenic hormone in the equivalent of fresh ovary used to cause these effects.

Summary. Histological observations were made on all animals reported by us in a previous publication.[1] It was observed that there was greater follicular development in the experimentals than in the controls of same age and litter. Follicular atresia was about the same. More interstitial cells were observed in the experimental ovaries. The uteri of the experimentals showed highly columnal epithelial cells. These observations seem to indicate some

[5] LONG, J. A., AND H. M. EVANS: *Memoirs of Univ. Calif.*, 6: 1. 1922.
[6] KOCH, F. C.: *Ann. Rev. Biochem.* 9: 327. 1940.

active substance other than estrone, estradiol or estriol in the ovary which has its effect through the hypophysis.

The authors wish to thank the Department of Zoology for permission to publish this paper as much of the work for it was done in that department under the direction of Dr. George Johnson, deceased.

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CHICK COMB METHOD FOR ANDROGEN ASSAY

IN THE APRIL NUMBER OF ENDOCRINOLOGY, there appeared three papers on the use of the chick comb for the bioassay of androgens.[1,2,3] These papers refer to work, utilizing the chick, published from several laboratories, and our contributions occupy a prominent place among these references. The most cautious of these three papers concludes that "in our hands and with a strain of baby chicks used by us, the baby chick androgen method is of doubtful value." The most positive report states that "the variation in the response of the chick comb to known *injection** of androgens is so large in either the Frank et alia method . . . that quantitative androgen assay of known or unknown samples is impossible with this method." Since these papers are in great measure a critique of our work, we propose to discuss them with particular reference to that part of their contents which is directly referable to our preliminary reports.

Prior to 1941 we published four preliminary or progress reports in the *Proceedings of the Society for Experimental Biology and Medicine.* The first of these[4] described a method of utilizing the 6-day-old chick for bioassay purposes and stated that "much of this study was occupied in eliminating pitfalls" and "we are not yet prepared to offer a characteristic curve of response." In the second of these[5] we stated that "the chief aim of the present work was to determine what changes in the experimental conditions previously employed must be made in order to effect increase in uniformity and sensitivity." One of these changes was the substitution of the 3-day in place of the 6-day-old chick. Our third and fourth papers[6,7] indicated the effect resulting from variation in the nature and volume of the vehicle used. In none of these preliminary or progress reports did we specify any details of acceptable bioassay technic nor did we present a dose response curve or equation for assay purposes. In January of this year we published a final progress report[8] in which we specified many of the details of our technic, presented a dose response equation effective in the dose range 0 to 50γ, and a standard error of estimate for such assays corresponding to 2.7γ or 11.3% (for the mean of the dose range). We also stated explicitly that this dose response equation is operative "under our working conditions," and that the reliability of the assay holds "provided these assays and experiments upon which the dosage response equation was based, constitute fair samples of the same total population." An analysis of the three articles in question reveals that the evidence contained in them, in so far as it applies to our studies, is based on working conditions which are different from those specified by us in a great many respects and ignores our final progress report completely.

Thus McCullagh and Guillet used 6- to 7-day-old chicks—we specified *3-day chicks* to minimize the spontaneous comb growth factor; they isolated "at least" their controls—we specify complete segregation of differently treated groups; their total dosage range is 0 to 20γ, ours 0 to 50γ, with the knowledge that we must restrict ourselves to the upper part of this

* Italics are ours, we use "application" only.

[1] DUFF, P. A., AND H. H. DARBY: *Endocrinology* 28: 643. 1941.
[2] MCCULLAGH, D. R., AND R. GUILLET: *Endocrinology* 28: 648. 1941.
[3] HOSKINS, W. H., G. W. BEACH, J. R. COFFMAN AND F. C. KOCH: *Endocrinology* 28: 651. 1941.
[4] FRANK, R. T., AND E. KLEMPNER: *Proc. Soc. Exper. Biol. & Med.* 36: 763. 1937.
[5] FRANK, R. T., E. KLEMPNER, AND F. HOLLANDER: *Proc. Soc. Exper. Biol. & Med.* 38: 853. 1938.
[6] KLEMPNER, E., F. HOLLANDER, AND R. T. FRANK: *Proc. Soc. Exper. Biol. & Med.* 44: 631. 1940.
[7] KLEMPNER, E., R. T. FRANK, AND F. HOLLANDER: *Proc. Soc. Exper. Biol. & Med.* 44: 633. 1940.
[8] HOLLANDER, F., E. KLEMPNER, AND R. T. FRANK: *Proc. Soc. Exper. Biol. & Med.* 46: 1. 1941.

range (at least above 10γ) in order to keep the *percentage errors* at a minimum; as a criterion of response, they employ the *ratio* of comb weight to body weight—we developed a body weight correction by the recognized statistical technic of multiple regression, based on both initial and final body weights, because we found that the simple ratio varied with the comb weight and therefore could not be used with maximum reliability; they correct for sex by a factor of 1.20 for females—we developed a correction for sex by multiple regression and which is weighted for the relative numbers of males and females in the group; they use a parabolic dose-response equation of their own, without stating how it was obtained—our dose-response equation is very different from this and was developed by multiple regression. The working conditions of Duff and Darby which differ from our own are as follows: age of chick is variously 4 hours, 1 day, and 7 days; the criterion of response is the ratio of comb and body weights; the mode of administration is "to the base of the comb by inunction" and also by injection; the dosage range by inunction is variously 0 to 18 and 0 to 80γ; no dose-response equation is given. Furthermore, they misquote our accepted mode of administration and cite data from our earliest paper[5] obtained under conditions (6-day chicks, treated for 10 days, dosage range 0 to 200γ) which we have long since discarded.

A careful scrutiny of the evidence offered by Hoskins et al. revealed that the *injection* technic was used in all but one assay, which involved only 20 chicks; age of chicks is 1 day; duration of treatment is 5 days; total dose in the one application experiment is 10γ; their criterion of response is also the ratio of comb to body weight, the latter being measured sometimes with and sometimes without evisceration. In short, the working conditions of these various authors bear but slight resemblance to those which we specified in our paper of January 1941.

We feel, therefore, that these three articles are misleading as regards our own work and that the attention of workers in the field should be drawn to this fact, so that they may reserve final judgment as to the value of our chick comb method until we have published our detailed report on the technic and its mathematical justification. The reliability of our assay method, both with crystalline androsterone and with urine extracts, is such as to make us feel that it should not be discarded on such inconclusive evidence—particularly since the technique is very much less cumbersome and expensive than that of the capon method.

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EFFECTIVENESS OF VARIOUS METHODS OF SEX HORMONE ADMINISTRATION FOR THE INDUCTION OF HEAT IN THE SPAYED FEMALE GUINEA PIG AND RAT*

MANY EXPERIMENTS have been reported on the effectiveness of hormone administration by other than the parenteral route. It is quite generally agreed that the oral route is least effective[1] while percutaneous application is often nearly as effective as subcutaneous injection. In a previous report[2] on the oral effectiveness of progesterone and pregneninolone in the spayed female guinea pig it was shown that 20 times the subcutaneous dose was required to elicit behavioral mating responses. Using the mating response characteristic of the guinea pig and rat as a criterion,[3,4] it was practicable to compare the two spe-

* This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

[1] ALLEN, E., F. L. HISAW AND W. U. GARDNER: Chap. VIII, Sex and Internal Secretions, Williams & Williams, Baltimore, 1939.

[2] SODERWALL, A. L.: *Endocrinology* 27: 840. 1940.

[3] YOUNG, W. C., E. W. DEMPSEY, C. W. HAGQUIST AND J. L. BOLING: *J. Lab. & Clin. Med.* 23: 300. 1937.

[4] BOLING, J. L., AND R. J. BLANDAU: *Endocrinology* 25: 359. 1939.

TABLE 1. COMPARISON OF HORMONES AND HORMONE ADMINISTRATION FOR THE INDUCTION OF HEAT

Guinea pig				Rat			
Quantity	No. animals	No. in heat	% in heat	Quantity	No. animals	No. in heat	% in heat
Progesterone, oral (oil)				*Progesterone, oral (oil)*			
1.0 mg.	—sufficient for induction of heat.[2]			5.0 mg.	4	4	100.0
				4.0 mg.	10	1	10.0
				2.0 mg.	3	0	0.0
				1.0 mg.	10	0	0.0
Percutaneous (95%)				*Percutaneous (95%)*			
1.0 mg.	6	6	100.0	2.0 mg.	5	5	100.0
0.5 mg.	8	7	87.5	1.0 mg.	10	5	50.0
0.25 mg.	8	6	75.0	0.5 mg.	4	0	0.0
0.10 mg.	10	4	40.0	2.0 (oil)	6	0	0.0
Progynon-B, oral (oil)				*Progynon-B, oral (oil)*			
250 R.U.	13	2	15.4	500 R.U.	6	5	83.3
25 R.U.	6	0	0.0	250 R.U.	12	0	0.0
10 R.U.	6	0	0.0	125 R.U.	6	0	0.0
Percutaneous (oil)				*Percutaneous (oil)*			
125 R.U.	9	8	88.8	250 R.U.	5	4	80.0
62 R.U.	5	5	100.0	125 R.U.	6	0	0.0
30 R.U.	5	0	0.0	62 R.U.	6	0	0.0
15 R.U.	5	0	0.0	48 R.U.	6	0	0.0
				25 R.U.	12	0	0.0
Pregneninolone, oral (tablet)				*Pregneninolone, oral (crystal)*			
1.0–2.0 mg.—sufficient for induction of heat.[2]				15.0 mg.	5	0	0.0
				10.0 mg.	9	0	0.0
				5.0 mg.	7	0	0.0
Percutaneous (95%)				*Percutaneous (95%)*			
5.0 mg.	4	0	0.0	5.0 mg.	9	0	0.0
2.5 mg.	3	0	0.0				
1.0 mg.	9	0	0.0				

cies in their sensitivity to various sex hormones when administered orally, percutaneously or subcutaneously.

Materials and methods. Spayed female guinea pigs and rats are properly conditioned if given 10 R.U. estradiol benzoate† subcutaneously in oil. The animals can be brought into heat if they receive subcutaneously 0.05 mg. (guinea pig) or 0.4 mg. (rat) progesterone† 48 or 72 hours later. These figures for subcutaneous injection make an excellent basis with which to compare experimental results. Sexually mature spayed females received either orally or percutaneously administered estradiol benzoate, progesterone or pregneninolone.† The hormones in sesame oil or crystalline form were fed in gelatin capsules. The hormones applied externally to the shaved skin were dissolved either in sesame oil or 95% alcohol. Shortly before and subsequent to receiving the inducing dose the conditioned animals were observed at hourly intervals for the display of sexual receptivity.

Results. Table 1 presents the tabulated results of the relative effectiveness of graded doses of the 3 hormones administered orally and percutaneously to the spayed female guinea pig and rat.

One of the results most apparent from the table is the greater sensitivity to hormone stimulation of the guinea pig when compared with the rat. The greater refractiveness of the rat to progesterone injected subcutaneously has previously been shown by Boling and

† The estradiol benzoate, in the form of Progynon-B, and progesterone, in the form of Proluton, were supplied by the Schering Corporation, Inc., Bloomfield, N. J., through the courtesy of Dr. Erwin Schwenk; the pregneninolone, in the form of Lutocylol, was supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J., through the courtesy of Dr. Ernst Oppenheimer.

Blandau.[4] Twenty times the injection quantity is required for the elicitation of heat responses in the guinea pig when progesterone is given orally in oil.[2] Oral administration is also effective in the rat; however, approximately 12 times more than the quantity necessary by injection must be administered when given by this route. When progesterone is dissolved in 95% alcohol its effectiveness is greater than per os but less than by the subcutaneous route. About 5 times the subcutaneous dose is required for both species, the guinea pig being the more sensitive. The same quantity effective percutaneously in alcohol was applied in sesame oil to the rat with negative results.

The greater effectiveness of the alcohol solution is in harmony with the observations of Klempner, Frank and Hollander[5] and others, that highly volatile solvents allow for a more direct absorption of the hormones through the skin. Soloway, Hansen and McCahey[6] have reported that oil is not absorbed beneath the stratum corneum of the skin so that selective diffusion of the hormone is required. This factor of differential absorption of solvents has brought about a preference for such solvents as alcohol, ether and benzine, for the percutaneous application of hormones.

Extradiol benzoate, administered per os, in oil, was likewise much less effective than by subcutaneous injection, 50 times the quantity being required. Application of this hormone to the shaved skin of the back is more effective than when administered orally. The guinea pig requires only 6 times the subcutaneous dose for proper conditioning; the rat about 25 times.

Pregneninolone when administered orally in tablet form to the guinea pig was nearly as effective as progesterone.[2] Successful results from feeding the crystalline form of progesterone to the guinea pig led us to attempt the same therapy with pregneninolone in the rat. This proved to be entirely ineffectual. Quantities as large as 15.0 mg. induced no responses. The percutaneous administration of pregneninolone to both species likewise evoked no behavioral reactions characteristic of normal mating behavior. Other investigators using this hormone have reported results quite at variance with one another. Courrier and Jost[7] were able to maintain pregnancy in castrate pregnant rabbits, whereas Pincus and Werthessen[8] were unsuccessful. The latter workers attempted this with intraperitoneal implantation of 15.0 mg. of pregneninolone; the former by oral and subcutaneous injections of higher doses. The difficulty of getting this substance into solution might explain the disparity of the results.

In all of these experiments the character of the heat responses as determined by the latent period, length of heat and intensity of mating behavior was within normal range.

Summary. The oral and percutaneous methods of administration of sex hormones to the spayed guinea pig and rat are much less effective than the subcutaneous route for the induction of mating responses. The rat shows the greater refractiveness to hormone therapy regardless of the route used in administration. Percutaneous application is more effective than oral administration, less effective than subcutaneous injection.

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[6] SOLOWAY, D., L. P. HANSEN AND J. F. MCCAHEY: *Proc. Soc. Exper. Biol. & Med.* 41: 547. 1939.
[7] COURRIER, R., AND A. JOST: *Compt. Rend. Soc. de Biol.* 130: 1162. 1939.
[8] PINCUS, G., AND N. T. WERTHESSEN: *Am. J. Physiol.* 124: 484. 1938.

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[1] Pages 341a, 344a are in the April issue; 341b is in the May issue

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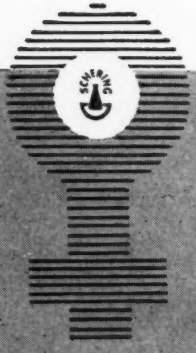

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